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EXCRETION OF GLYCOGENIC CORTICOIDS AND OF 17-KETOSTEROIDS IN VARIOUS ENDOCRINE AND OTHER DISORDERS

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THERE have been a number of methods of estimating the function of the adrenal cortex in clinical disorders. First has been the observation of clinical symptoms and signs and the grouping of these into the classical syndromes associated with gross diseases of the adrenal cortex. Difficulties have arisen in that many cases do not possess all the features of the well established disease and terms such as Addisonism and hypoadrenalism have been coined to describe those clinical states in which the evidence for hypofunction of the adrenal cortex is often largely inferential. On the hyperfunctional side the association of virilism with hyperplasia and tumors of the adrenal cortex has resulted in the tendency to attribute all cases of simple hirsutism to hyperfunction of this gland.

Second, the observation at autopsy or at operation of gross and microscopic morphological changes in the gland has been used. It was by this method that the classical syndromes were established. The method cannot of itself, however, detect changes in function which may occur without gross morphological change and the observation in clinical cases is usually a single one often made after the death of the patient. Also the association of changes in lipid content directly with functional change cannot be

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done on morphological grounds alone; for example, a depletion of the lipids of the gland does not necessarily indicate functional exhaustion.

Third has been the use of various metabolic indications such as disturbances of electrolyte and carbohydrate metabolism, which have been shown experimentally to be influenced by adrenal cortical function. These are indirect measurements which may be affected by other glands and other factors than the function of the adrenal cortex.

Fourth has been the measurement in blood or urine of the compounds secreted by the adrenal cortex or of their metabolic products. The so-called total neutral 17-ketosteroids have been used for this purpose and have been shown to be low in cases of Addison's disease and high in cases of adrenal tumor. They have also been extensively used to indicate changes in adrenal function of a lesser degree. This determination suffers from the disadvantage of all measurements of excretion products in that a number of factors beside the actual rate of secretion by the gland affect their rate of excretion. 17-ketosteroids are derived not only from the adrenal cortex but, in the male, from the testis. The fact that there are a large number of them and that changes in individul members of the group may occur and have significance apart from the total rate of excretion has been well shown by Dobriner (2) and his group.

Fifth the measurement of biologically active corticoids. This has been done in the adrenalectomized animal, either by life maintenance, protection against stress, or by measurement of the activity of these substances in carbohydrate metabolism. This method suffers also from the disadvantage mentioned above in that the relation of secretion of the gland to level of excretion of the material measured, may be affected by many factors. So far as is known, highly active substances of this type are derived only from the adrenal cortex.

Finally, chemical methods involving colorimetric procedures have been devised for assaying adrenal cortical steroids. The methods of Talbot and of Heard and Sobel are based upon the reducing properties of adrenal steroids, that of Lowenstein, Corcoran and Page, on periodate oxidation of the primary alcohol group at C_{21} which yields formaldehyde which can be measured colorimetrically. These methods may include metabolites of adrenal hormones which are not active in the biological assay and give much higher values than the bioassay.

It has become apparent in recent years through the work of investigators that the adrenal cortex has a variety of functions. The substances so far derived from it may be roughly divided into three groups: (a) the compounds which act mainly on electrolyte metabolism, (b) the compounds which act mainly on protein and carbohydrate metabolism, (c) a group

which has properties of the sex hormones. In this group fall the androgens which also affect protein metabolism, the estrogens and progesterone. An increased or decreased secretion of these substances might then lead to qualitatively different states of hyper or hypo function of the adrenal depending upon the relative amounts of the various substances secreted. The qualitative and quantitative variations in function thus produced could lead to a variety of metabolic and clinical disorders some of which may not have been previously associated with disturbances of adrenal function, and others of which account for the variation in the clinical and metabolic picture in cases grouped under the headings of Addison's disease, Cushing's syndrome, adreno-genital syndrome, etc. Qualitative variations in cases falling into the category of Cushing's syndrome and the possible relationship of these to variations in adrenal function, have recently been discussed by Kepler and Sprague (8).

It seemed of interest to apply a recently developed biological method of determining adrenal steroids possessing activity in carbohydrate metabolism to the determination of urinary corticoids in various clinical disturbances and to compare the variations in this type of activity with the variations in total 17-ketosteroids. One has in this biological assay a method of measuring, even though indirectly, one type of adrenal cortical function. Neither the group of substances purely active in electrolyte metabolism nor the androgens, etc., affect the assay in the quantities in which they are likely to be found in the urinary extract used. The biological method developed by Venning, Kazmin and Bell (12), based upon the ability of adrenal substances to cause a deposition of glycogen in the liver of the adrenalectomized fasted mouse, was used in these studies.

It has previously been shown by several authors that there are present in normal human urine substances that have the properties of adrenal cortical compounds. Anderson, Haymaker and Joseph (1), reported that extracts from the blood and urine of patients with Cushing's syndrome were effective in prolonging the life of adrenalectomized rats. Weil and Browne (14), found an increased excretion of substances active in the Selye-Schenker cold exposure test in the urine of a patient suffering from Cushing's syndrome, in some cases of hypertension, as well as after injury, infections and surgical operations. Dorfmann, Horwitt and Shipley, (3) were unable to detect cortin-like material using a cold exposure test in fourteen out of fifteen samples of urine from seven patients with Addison's disease, whereas urine from normal individuals did show activity in this test. The excretion of 17-ketosteroids and glycogenic corticoids in normal individuals has been discussed in a previous paper (13). The present one deals with the findings in various endocrine disorders. Preliminary reports

of our investigations have been presented at the Conferences on Metabolic Aspects of Convalescence, 1942–46, held under the auspices of the Josiah Macy, Jr., Foundation.

METHODS

A 48-hour specimen of urine was collected on successive days. No preservative was used but the urine was kept cold after collection. The volume was measured, 100 cc. removed for the 17-ketosteroid determination, and the remainder was acidified to pH 1.0, extracted and assayed for glycogenic corticoids according to the method of Venning, Kazmin and Bell (12). The activity is expressed in terms of glycogenic units excreted per twenty-four hours, one glycogenic unit being equivalent to the biological activity contained in one microgram of 17 hydroxy-11 dehydro corticosterone. Colorimetric measurements of the 17-ketosteroids were performed according to the method of Holtorff and Koch (7), and a correction factor for non-steroidal chromogens was applied.

RESULTS AND DISCUSSION

Normal Individuals

These values have been reported previously (13) but are listed in Table 1 for comparison with the results in abnormal conditions. The excretion of both 17-ketosteroids and of glycogenic corticoids is found to be higher in the male than in the female adult. While little or no glycogenic activity could be detected in the urine of the new-born male infant, by the age of 2½ years there was already present an amount within the range found in the urine of the normal adult. The values for 17-ketosteroids and for glyco-

TABLE 1. EXCRETION OF 17-KETOSTEROIDS AND GLYCOGENIC CORTICOIDS IN THE NORMAL INDIVIDUAL

Sex	No. cases	Age	17-Ketosteroids		Corticoids	
			range	average	range	average
		years	mg.	mg.	gl. units	gl. units
Female	12	21–48	7–18	11	25–55	39
Female	2	74–76	6–8	—	26–38	—
Male	12	20–51	10–25	17	40–85	60
Male	2	77–81	10	—	20–37	—
Male	6	1–4 days	—	—	0–15	—
Male	2	3	2	—	40–42	—
Male	1	5½	5	—	53	—
Male	1	13	6	—	55	

genic corticoids did not necessarily parallel each other even in adults. In the case of young children the corticoids reached adult levels long before the ketosteroids. It has also been reported by Venning (11) that in late

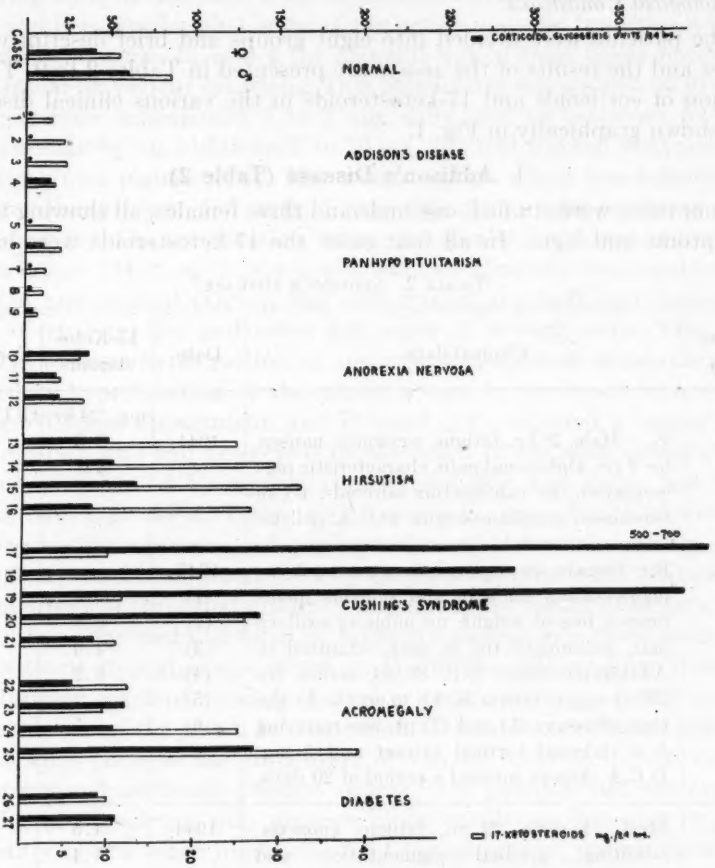

FIG. 1. Excretion of Glycogenic Corticoids and 17-Ketosteroids in Various Endocrine Disorders.

pregnancy the glycogenic corticoids reach very high values whereas the true 17-ketosteroids do not rise. It must be borne in mind that the 17-ketosteroids are excreted in very much larger amounts than the corticoids, milligrams of 17-ketosteroids as against micrograms of corticoids. The corticoids are labile substances and their degree of destruction or conversion into other substances in vivo is unknown; the excretion may repre-

sent only a small fraction of the total amount elaborated in the gland itself. This, however, still permits interpretation in terms of relative changes from the normal level in each.

Pathological Conditions

The patients were divided into eight groups and brief descriptive histories and the results of the assays are presented in Tables 2 to 9. The excretion of corticoids and 17-ketosteroids in the various clinical disorders are shown graphically in Fig. 1.

I. Addison's Disease (Table 2)

Four cases were studied, one male and three females, all showing typical symptoms and signs. In all four cases the 17-ketosteroids were low. In

TABLE 2. ADDISON'S DISEASE

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
1	W.—Male, 28 hr., fatigue, weakness, nausea for 2 yr., abdominal pain, characteristic pigmentation, no calcification adrenals, no tuberculosis, maintained with D.C.A. pellets.	1944	2.3 3.8	<10 —
2	B.—Female, 56 yr., weakness, nausea 2 yr., pigmentation on skin and mucous membranes, loss of weight, no pubic or axillary hair, pulmonary tbc in past, admitted in Addisonian crisis. B.P. 80/40, serum Na 130 m.eq./1, serum K 6.8 m.eq./1. At the time of assays (1) and (2) pt. was receiving 5 cc. adrenal cortical extract and 5 mg. D.C.A. Assays covered a period of 20 days.	1945 (1) (2) (3) (4) (5) (6)	 2.5 6.8 4.3 6.2	 — 15 — <10 <10 <10
3	McK.—Female, 29 yr., fatigue, anorexia, vomiting, gradual pigmentation and amenorrhea 7 mos., increased glucose tolerance, insulin sensitivity test showed failure of B.S. to rise, B.P. 90/60, serum Na 134 m.eq./l. Maintained on D.C.A.	1944 1945	4.5 4.4 — 5.2	<10 <10 <10 <10
4	D.L.—Female, 39 yr., headache, nausea, weakness, fatigue, pigmentation 2 yr., anorexia, loss of weight, tbc. hip at age 7, no calcification of adrenals. Serum Na 130 m.eq./l., serum K 5.3 m.eq./l. B.P. 98/66.	1945 May June Nov.	 1.9 4.4	 23 10 22

cases 1 and 3 (patient of Dr. E. H. Mason), less than the amount of glycogenic corticoid activity detectable by the method was present. In case 2, in the second assay, 15 glycogenic units were detected, the patient was receiving daily at this time 5 cc. of adrenal cortical extract intramuscularly and it is possible that some of the active material from this was excreted in the urine. In case 4 the amounts varied from below normal to low normal on various occasions. This patient was maintained on desoxycorticosterone sublingually 5 to 7 mg. daily, and on occasions received intramuscularly, an additional 5 to 10 mg. She had marked weakness and definite brown pigmentation. Her serum sodium which was consistently low, remaining about 135 m.eq./l, was difficult to raise to normal with DCA therapy. On one occasion, when the dose of DCA was reduced, it fell as low as 124 m.eq./l. She responded best clinically to a combination of DCA and cortical extract. She menstruated regularly and showed no signs of virilism. She had never had signs of hypoglycemia. That it is possible to have hypofunction of one aspect of adrenal metabolism and normal or hyperfunction of the others is seen in two cases reported recently. Wilkins, Fleischmann and Howard (15), reported a case of a $3\frac{1}{2}$ year old boy who had precocious development of the male accessory sex organs accompanied by pigmentation of the skin and gums, low blood sodium and high NPN. Talbot (10) described a case of a child with the electrolyte disturbances and pigmentation of Addison's disease, with normal oxysteroids as determined by his chemical procedure. This patient also had premature sexual maturity and markedly raised 17-ketosteroids. Talbot has suggested a division of Addison's disease into those cases with and without hypoglycemic crises. It is questionable whether these are qualitatively different types of disease or represent stages in the process of destruction of the gland. In connection with Addison's disease one may ask at what point in the diminution of adrenal function do the various symptoms and metabolic changes appear. It is possible that one type of function of the adrenal may decrease more rapidly than another or independently of the other functions. We have at present unfortunately no method of measuring the purely electrolyte regulating function of the adrenal by means of any excretion products.

The present findings, together with those of Dorfman, Horwitt and Shipley (3) and cases cited by Forbes (5), indicate that the biologically active corticoids are very low in the majority of cases of Addison's disease. Further cases will have to be studied to determine whether there are variations dependent upon the stage or severity of the syndrome, or whether within the syndrome qualitative variations in the type of adrenal deficiency can be detected.

TABLE 3. PANHYPOPITUITARISM

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
5	B.—Male, 48 yr., fatigue, pallor, skin dry and thin, absence of facial, pubic and axillary hair, impotence, testicular atrophy, headache, craniopharyngioma removed in 1924. B.M.R. −25%.	1945	3.7	<10
			4.5	<10
			3.6	<10
6	T.—Male, 32 yr., well nourished, hypersensitivity to cold, fatigue, headache, dizziness, one hypoglycemic crisis, absence of pubic and axillary hair, testicular atrophy and impotence, enlargement of sella. B.M.R. −45%. Therapy: implantation of testosterone propionate pellets.	1944	3.3	17
		1945	6.0	<10
7	N.—Male, 41 yr., fatigue, absence of body hair, anemia, B.M.R. −22%. Enlargement of sella.	1945	3.5	<10
			2.6	<10
8	H.—Female, 45 yr., pallor, weakness, anorexia, some loss of weight, amenorrhea since last pregnancy (23 yrs.), hemorrhage following delivery at that time, anemia, absence of axillary and pubic hair, hypoglycemic crises in 1940,—none since then. B.P. 84/60, B.M.R.-30%. In 1944 received 25 mg. testosterone propionate daily. At other times 40 mg. methyl testosterone daily.	1940	1.5	
		1941	3.9	
		1942	4.2	
		1944	13.4	<10
		1945	5.4	<10
			2.0	<10
9	R.—Female, 47 yr., cachectic, lethargic at time of admission 1944, weakness, absence of axillary and pubic hair, amenorrhea since last pregnancy 17 years ago after severe postpartum hemorrhage. B.M.R. −30%.	1944	1.4	<10
			2.3	<10
			1.5	10
		1946	1.4	<10

II. Panhypopituitarism (Table 3)

Five cases were studied, three males and two females. All showed many signs of pituitary hypofunction. Of the three males, Case 5 had had a craniopharyngioma which had been removed 22 years prior to the study and cases 6 and 7 showed enlargement of the sella turcica. Cases 8 and 9, both females, gave a history of hemorrhage at the time of labor 23 and 17

years respectively, before study. In all these cases the glycogenic corticoids were markedly low on repeated determinations. In case 8 it is of interest to note that when she had an acute pyelitis with fever the corticoid value was 27 units, a low normal value. This is, however, much lower than the value one would find in a normal individual with an acute infection. As soon as the fever subsided that value fell to less than 10 units. It was raised to 32 units by the administration of pituitary adrenocorticotropin, falling to less than 10 units again on cessation of the injections. This response indicates that the adrenal is still capable of responding to the pituitary after years of hypofunction, and that there probably is in this case a small amount of pituitary tissue left which responds to stress but the quantitative response, as reflected in the urinary corticoids is much less than normal. Since it is quite possible, with partial destruction of the pituitary or adrenal, leading to symptoms of panhypopituitarism or Addison's disease, that the glands are still capable of increasing their secretions under acute stress, the finding in acute infection or after trauma of a value within the limit found in the healthy normal adult, does not necessarily indicate that there is no deficiency.

It is of interest that these individuals did not show marked evidence of electrolyte disturbance in spite of the similarity of their glycogenic corticoid excretion to that found in Addison's disease. This may be further evidence of the possibility of dissociation between two types of adrenal hypofunction. In spite of the low urinary corticoids these individuals ordinarily had normal fasting blood sugars. Case 8 had had repeated hypoglycemic crises in 1940 but had had none since in spite of the occurrence of several infections during which she did not eat for several days and came into the hospital dehydrated. Her blood sugar on two of these occasions on admission was 84 and 78 mg. per cent. As indicated, a small amount of glycogenic corticoids appeared in the urine during the acute infection. Case 6, now followed over eight years, has had only one hypoglycemic crisis, this a severe one in 1944, with semiconsciousness for several days, following an upper respiratory infection. Yet he had been a telephone linesman out in all weathers and eating when he could for years before his one episode of hypoglycemia. This raises the question again at what level of pituitary and adrenal hypofunction does such a disturbance as hypoglycemia arise. Is it below that at which glycogenic corticoids are detectable in the urine by this method? There are probably also other factors entirely outside the endocrine system which may elicit the actual hypoglycemia. One of these could be failure to eat adequately due to the extreme lethargy which often accompanies this disease. However, none of the cases described above, with the exception of Case 9, (patient of Dr.

C. Fullerton), lost much weight or were cachectic in appearance.

In these cases of panhypopituitarism, therefore, the values for glycogenic corticoids are very low and are comparable to those seen in Addison's disease. Occasionally small amounts were present and in one case an acute infection caused the appearance temporarily of glycogenic corticoid activity in the urine. The 17-ketosteroids were low but not as low as in the cases of Fraser, et al. (6).

These cases were all treated with thyroid extract and testosterone or methyl testosterone and responded satisfactorily to the therapy.

TABLE 4. ANOREXIA NERVOSA

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
10	B.—Female, 24 yr., wt. 84½ lbs., loss of weight, asthenia, amenorrhea 2 yr., axillary and pubic hair present, anemia, psychological difficulties. B.P. 100/60. B.M.R. −26%.	1945	7.1 6.4	— 36
11	S.—Female, 37 yr., poor appetite, emaciated, lost 44 lbs. from 1928 to 1932, remained between 63–80 lbs. until 1942. Appetite improved and has gained 27 lbs. from 1942–1945. Amenorrhea 13 yr. Axillary and pubic hair present, psychological difficulties. Originally diagnosed as Simmonds' disease, diagnosis changed in 1942 to anorexia nervosa. Lowest B.M.R. in 1932 −27%.	1945	4.0 3.5	17
12	F.—Female, 25 yr., wt. 92 lbs., asthenia, loss of weight, anorexia, headaches, emotional and family difficulties. B.P. 107/94, B.M.R. −21% in 1935. No physical signs of hypothyroidism. In 1938 serum cholesterol = 484 mg.%; in 1944 = 287 mg.%.	1944	7.2	20

III. Anorexia Nervosa (Table 4)

The differential diagnosis of this condition from that of panhypopituitarism due to organic disease of the pituitary is admittedly difficult. It is generally diagnosed in young individuals with no evidence of pituitary tumor, no history of difficult labor or hemorrhage at labor, no hypoglycemic crises, a retention of pubic and axillary hair, and a history of marked

emotional disturbance with loss of appetite or refusal to eat adequately. Accompanying the loss of weight, there is a low B.M.R. which is followed by amenorrhea and other evidences of pituitary hypofunction. The pituitary hypofunction has been regarded as occurring as a consequence of the malnutrition. Three cases which are regarded as typical as regards history and physical findings were studied. The 17-ketosteroids were low but in general not as low as those in the cases of panhypopituitarism described in the previous group. Glycogenic corticoids were present in detectable amounts in all three cases, ranging from 17 to 36 glycogenic units, values

TABLE 5. HIRSUTISM (SIMPLE)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
13	W.—Female, 30 yr., obesity, hirsutism face only, irregular menses 6–7 weeks for 1½ yr., previously regular, no clitoral hypertrophy, scalp hair thinning, no true apical baldness.	1945 Mar. May	 26.2 23.1	 49 52
14	M.—Female, 30 yr., excessive hair on face 16 yr., irregular menses, dysmenorrhea, gained 150 lbs. between ages of 12 and 15 yr. due to increased appetite. In 1939 placed on 1200 cal. diet, lost 150 lbs. over 10 mo. Cholelithiasis in 1940, no clitoral hypertrophy, pregnandiol present from 16th to 27th day of a 28 day cycle. Total amt. 26 mg.	1945	29.7	54
15	L.—Female, 16 yr., facial, chest, and abdominal hirsutism 2 yr., hair growth on arms and legs increased, scalp hair falling out, acne, no clitoral hypertrophy, irregular menses 6–8 weeks, pregnandiol present in luteal phase.	1945	27.8 37.0	65
16	M.—Female, 54 yr., excessive hair on face, markedly obese, amenorrhea 5 mos., hot flushes. Hypertensive cardiovascular disease. B.P. 180/130. Generalized arteriosclerosis. Osteoarthritis of phalanges, fibromyoma ovary, uterine fibroids, at operation adrenals explored, right adrenal somewhat enlarged.	1945	27.0	38

which are normal or slightly below normal. Both in the group of panhypopituitarism with organic lesions and this group, there is an indication of a quantitative difference in degree of reduction of adrenal function, in relation to varying degrees of reduction of pituitary function due either to more or less destruction by an organic lesion or to varying degrees of functional impairment as a response to malnutrition or some other influence outside the pituitary itself.

IV. Hirsutism—Simple (Table 5)

Four cases of simple hirsutism are presented. None of these patients showed any signs of Cushing's syndrome or other signs of virilism. In two of them, although the menses were irregular, a luteal phase was present as evidenced by the presence of pregnandiol at the expected time in the cycle. The 17-ketosteroids were definitely raised in all four cases, while the glycogenic corticoids were normal or only slightly above normal. Comparing the 17-ketosteroid and glycogenic corticoid values in these cases with those of Cushing's syndrome, it is obvious that these two values can vary independently. Thus the 17-ketosteroids may give evidence of an increased adrenal function while the corticoids remain normal and the reverse condition may also occur.

V. Cushing's Syndrome (Table 6)

As has been mentioned, Anderson, Haymaker and Joseph first showed the presence of life-maintaining corticoids in the blood and urine of a case

TABLE 6. CUSHING'S SYNDROME

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
17	A.—Female, 33 yr., weakness, hirsutism, amenorrhea since last pregnancy 13 mo., plethoric appearance, tendency to bruise, ecchymosis of both lower legs, osteoporosis of vertebrae, large but normal sella. B.P. 150/100. Glucose tolerance test—Fasting B.S. 84; 30 min. 172; 60 min. 152; 120 min. 148; 180 min. 119 mg. %. Insulin resistant. Operation—left adrenal containing benign adenoma size of walnut removed. Autopsy showed atrophy of right adrenal with small cortical adenoma.	1944	10.0 8.1	700 500

TABLE 6. (*Continued*)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
18	C.—Female, 42 yr., headache, increasing facial hirsutism and obesity 12 yr., weakness, drowsiness, irregular menses, plethoric appearance, oesteoporosis, hypertrichosis of face and trunk. B.P. 230/135. Glucose tolerance test—fasting B.S. 126; 30 min. 199; 60 min. 262; 120 min. 175; 180 min. 130 mg.%. X-ray showed no abnormality of sella turcica.	1945	20.4 22.2 23.6	340 212 316
19	C.—Male, 11 yr., Symptoms had developed within a year, plethoric appearance, obesity with typical moon face, large hump in the upper dorsal region, moderate osteoporosis especially in lumbar spine, definite striae of the skin, normal genital development with some scant pubic and axillary hair. The sella was normal in size. Blood sugar 116 mg.% 1½ hr.p.c. Exploration of the adrenals showed no distinct abnormality.	1946	11.5	386 218
20	S.—Female, 39 yr., increasing facial hirsutism and acne with plethoric appearance 6 mo. Raised B.P. and protuberance of abdomen noted 1 year previously. Glucose tolerance tests of diabetic type. Relatively little osteoporosis. Sella turcica normal. Operation 2 weeks prior to assays—right adrenal removed—2½ times normal size—contained adenoma which had obliterated medullary space.	1946	26.1	137
21	F.—Male 27 yr., obesity and plethoric appearance 15 yr., hemorrhage in retinae. B.P. 210/140, mild osteoporosis of spine and pelvis. Glucose tolerance was within normal range. X-ray therapy in May 1943 with no improvement. Left adrenal and part of right adrenal removed in October 1943. No abnormality found. Still shows clinically the same evidences of Cushing's syndrome as before operation.	1946	11.0	42

of Cushing's syndrome. In 1939 Weil and Browne found by the cold exposure test an amount of urinary corticoid above normal in a male with typical Cushing's syndrome. In this same patient in whom the symptoms and signs of the disease disappeared, there was found a normal value by the cold exposure test in 1941 and again in 1945 by the glycogen assay. Eggleston and Dobriner (4) reported urinary corticoids in three cases of Cushing's syndrome; two showed markedly raised values, the third, normal values.

Five cases are presented in this study. In case 17 (Dr. Fuller Albright's patient), the glycogenic corticoids were very high, 500–700 units per twenty-four hours. The 17-ketosteroids were not raised. Assays on this patient's urine were also performed in Dr. Dobriner's and Dr. Albright's laboratory, both groups found values markedly above normal, Case 18 (a patient of Dr. E. H. Mason), was a typical case of several years duration. Operation some years previously at the Mayo Clinic had revealed no gross abnormality of the adrenals. The 17-ketosteroid values were slightly above normal, the glycogenic corticoids were markedly raised, varying from 160 to 340 units per 24 hours. Case 19 (Dr. E. Perry McCullagh's patient), a boy of 11 years, in whom the symptoms were of recent development, had 17-ketosteroids within normal range for his age. The corticoids were greatly increased, 218–386 glycogenic units. Case 20 (Dr. E. Perry McCullagh's patient), a woman of 39 years with typical appearance of Cushing's syndrome—most of her symptoms and signs had developed within the last year—showed an increased excretion of corticoids and 17-ketosteroids. In case 21 evidences of Cushing's syndrome had been present for 12 years. His left adrenal and part of the right adrenal had been removed in 1943, and although he was in much better condition in 1946 than before the operation, he still showed clinically the same evidences of Cushing's syndrome as before. The 17-ketosteroids and corticoids were normal.

From the present findings and those of others, it is apparent that cases of active Cushing's syndrome show high values for glycogenic corticoids. In certain patients in whom the process has been arrested or has become inactive, normal values are found. The values may be increased either with or without the presence of an adrenal tumor. The rise in glycogenic corticoids is not necessarily accompanied by a rise in 17-ketosteroids.

It is of interest that in case 17 the glucose tolerance curve was only slightly impaired though she was insulin resistant, whereas the glucose tolerance curves in cases 18 and 20 were definitely impaired. The glycogenic corticoids were higher in the first case. It is also true that in other conditions in which the urinary glycogenic corticoids may be much in-

creased, for example in pregnancy and after trauma and surgical operations, a raised urinary corticoid value and a normal glucose tolerance curve may coexist in the same patient at the same time. This is another illustration of the fact mentioned in the introduction that a metabolic test such as glucose tolerance is influenced by many factors and cannot be used as an index of any single glandular function.

While we have not had the opportunity of studying any cases of adrenal cortical tumor, those that have been reported in the literature usually show markedly increased 17-ketosteroids and Eggleston and Dobriner (4) have reported that the glycogenic corticoids were also increased in two cases of adrenal cortical carcinoma.

VI. Acromegaly (Table 7)

Four cases of acromegaly are presented. Cases 22 and 24 were of long standing and both showed normal values for glycogenic corticoids. The 17-ketosteroids were normal in case 22 and only slightly increased in case 24. The latter case had shown a marked impairment of sugar tolerance existing for several years. In 1945 he developed symptoms of polyuria and polydipsia. The symptoms and the blood glucose values were controlled by diet alone, although lately he has required small doses of insulin. This is not characteristic of the type of carbohydrate disturbance frequently seen in acromegaly which is usually insulin resistant. Case 23

TABLE 7. ACROMEGALY

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
22	S.—Female, 54 yr., gradual enlargement of hands, feet and face over a period of 15 years, headaches, insomnia, fatigue increasing 5 years. X-ray shows enlarged pituitary fossa with calcification of pituitary gland. Glucose tolerance test impaired. B.P. 210/90. X-Ray therapy in 1944.	1945	12.7	26
23	D.—Female, 32 yr., severe headaches and amenorrhea 3 years, enlargement of hands, feet and face 1½ years. X-ray showed enlarged sella pituitary tumor. Radiation of pituitary in 1944. Chromophobe adenoma of pituitary removed in 1945.	1945	11.8	62

TABLE 7. (*Continued*)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
24	J.—Male, 39 yr., marked prognathism, extremely large hands, feet and tongue. Received deep X-ray in 1932 for two years which arrested progression of symptoms. Diabetes mellitus controlled by diet. Glucose tolerance curve—Fasting B.S. 172; ½ hr. 314; 1 hr. 432; 2 hr. 312; 3 hr. 196 mg.%. Not insulin resistant.	1945 1946	26.3 24.0	53 40
25	G.—Male, 28 yr., fatigue and somnolence 2 years. Pain in neck, head and face 2 years. Decreased sexual function 8 months. Prognathism, terminal tuffing of phalanges. X-ray showed marked enlargement of sella. Glucose tolerance test normal. B.M.R. −11%.	1946	37.6 28.6	134

(a patient of Dr. W. Penfield), with acromegaly of relatively short duration, also showed normal values for glycogenic corticoids, and had received irradiation to the pituitary region in 1944. Case 25 (a patient of Dr. W. Penfield), an untreated case of recent origin, showed glycogenic corticoids of the order of 134 units, which is definitely raised. The glucose tolerance test was normal in this case. The 17-ketosteroids were also increased.

In this group of cases there is no correlation between the level of glycogenic corticoids and the disturbance of carbohydrate metabolism. However, signs and symptoms of the disease can exist without evidence of increased adrenal cortical function as measured by the glycogenic corticoids. The cases are too few to draw definite conclusions.

VII. Diabetes (Table 8)

Several cases of diabetes have been studied and all have shown normal values. The two cases presented are of interest in that their diabetes was difficult to control. Case 26 (patient of Dr. E. H. Mason) readily developed acidosis. The urine was collected following a period of acidosis when large amounts of insulin were being administered, and hypoglycemia was present part of the time. The corticoids were normal. Case 27 (patient of Dr. W. de M. Scriver) showed signs of increasing insulin resistance and the

TABLE 8. DIABETES

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
26	B.—Female, 27 yr., diabetes since 1929. Develops acidosis readily. Admitted to hospital in acidosis and ketosis. B.S. 335 mg.%. CO_2 18.8, vols.%. Received a total of 35 units P.Z. insulin, and 128 units C.Z. insulin. Urine collected for the next 48-hours for corticoids assay. Jan. 25—received a total of 56 units C.Z. insulin. B.S. a.c. 37 mg.%; p.c. (4 hrs.) 46 mg.%—no glucosuria but acetonuria present—insulin reaction in p.m. Jan. 26—insulin reaction in afternoon, no glycosuria.	1945	10.0	45
27	L.—Male, 59 yr., diabetes since 1934, insulin requirements 28 units daily. Has never been in diabetic coma. 3 weeks previously became confused and ran car into ditch. 3 days after accident patient vomited several hours, admitted to hospital in acidosis. B.S. over "1000." Since that time diabetes has proved impossible to control and patient has showed signs of increasing insulin resistance. Corticoid assay done 5 weeks after accident. Receiving 8 units P.Z. insulin and 32 units C.Z. insulin. B.S. a.c. 307; p.c. 358 mg.%. No glycosuria.	1946	10.8	54

diabetes was difficult to control. At the time of assay the blood sugar was above 300 mg. per cent. The 17-ketosteroids and corticoids were found to be normal.

VIII. Miscellaneous Cases (Table 9)

Case 28 was one of premature sexual maturity. There was no evidence of gross enlargement of the adrenal in the retrograde pyelogram but no retroperitoneal air injection or exploration of the adrenals was done. By pneumoencephalogram no evidence of intracranial lesion could be detected. The 17-ketosteroids were raised for a child of this age but the urinary corticoids were within normal limits. Case 29 was one of marked undergrowth and development in a child of $2\frac{1}{2}$ years. There was no x-ray evidence of gross lesion of the pituitary. The diagnosis of pituitary dwarf-

ism at this age is difficult but, as no obvious cause for her undergrowth was found, this diagnosis was tentatively made. The 17-ketosteroids and corticoids were within normal limits. The history of rapid growth in case 30 plus the evidence of the enlargement of the sella would suggest the presence of a pituitary tumor with active secretion of the acromegalic gigantism type. A hypofunctional state is indicated, however, by the presence of a slow pulse, a basal metabolic rate of −32 per cent, low blood corticoids, disappearance of body hair, and the delayed epiphyseal closure. This evidence suggests that the tumor is a non-functioning one and has by its enlargement destroyed the tissue of the pituitary so that the individual is at present suffering from panhypopituitarism. It is possible that the output of growth hormone was temporarily increased as the tumor began to grow. Case 31 has been diagnosed as Addison's disease because of the weakness, typical pigmentation, including mucous membrane pigmentation, and lowered blood pressure. The condition had not responded to DCA therapy over some time. It was obvious that he had an enlarged firm liver and there were other evidences of cirrhosis. The chest x-ray suggested the presence of neoplastic metastases but a primary lesion was not detected in the gastro-intestinal or urinary tract. He was suffering from marked malnutrition and had had anorexia, nausea and vomiting for some time. The impaired glucose tolerance curve was against a diagnosis of Addison's disease. Hemochromatosis was suggested; the pigmentation in the skin was melanin but cases of hemochromatosis are reported in which this has been found. The 17-ketosteroids were low but the corticoids were

TABLE 9. MISCELLANEOUS CASES

Case No.	Clinical data	Date	17-Ketosteroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
28	PRECOCIOUS SEXUAL DEVELOPMENT.	1945		
	A.—Male, 2½ yr., height 43¼ in., weight 47 lbs. Rapid physical and precocious sexual development began at age 1, testes and secondary sex organs adult size, axillary and pubic hair present, acne, deep voice.	May	12.5	55
		Nov.	10.7	41
		1946		
		July	5.6	42
29	PITUITARY DWARFISM. Female, 2½ yr., height 29¾ ins., poorly developed, under nourished and under weight. "Growth arrest" lines in long bones.	1946	3.2	48

TABLE 9. (*Continued*)

Case No.	Clinical data	Date	17-Keto-steroids	Corticoids
			mg./24 hr.	Gl.U./24 hr.
30	CHROMOPHOBE ADENOMA PITUITARY. J.—Male, 24 yr., has grown 3¼ in. and gained 100 lbs. in last 5 yr. Headaches 3 yr. Blurring of vision 1½ yr. Dizziness. Loss of body and axillary hair. Normal pubic hair. Enlarged sella and probable chromophobe adenoma. B.M.R. −33%. Glucose tolerance—Fasting B.S. 57; ½ hr. 117; 1 hr. 75; 1½ hr. 52; 2 hr. 68; 2½ hr. 74 mg.%.	1946	9.5	15
31	HEPATIC CIRRHOSIS. S.—Male, 62 yr., weakness 1½ yr. Loss of weight, drowsiness, vomiting, melanin pigmentation of skin and oral mucosa, enlarged firm liver, impaired liver function, raised plasma globulin, serum Na 141 m.eq./l. B.P. 95/60, impaired glucose tolerance, no response to D.C.A. therapy.	1945 June Sept. 8 Sept. 18 Oct. 12	 7.0 7.5 8.4	 44 47 117 49
32	HYPERTHYROIDISM. G.—Female, 49 yr. Thyroidectomy in 1936 for nodular toxic goiter. 3 years later regrowth of thyroid. 1946 B.M.R. +48%, amenorrhea since last pregnancy 1934. Weakness, nervousness, loss of weight. No axillary hair, slight pubic hair. Brownish pigmentation of skin,—none on mucous membrane. Cholesterol 106 mg.%. Serum Na. 141 m.eq./l.	1946	6.7	55
33	HYPOGONADISM. I.—Male, 40 yr. Weakness 8 yrs. Hot flushes 4 years. Occasional nausea and vomiting. Absence of facial, body and axillary hair. Scant pubic hair. Skin pale, does not tan. Testes small and soft. No libido.	1946	6.7 4.1	61
34	CHRONIC ASTHENIA. C.—Male, 44 yr. Fatigue, weakness, nervousness 15 yrs. Unable to work, underweight. B.M.R. −15−−25%. Sensitive to cold. Genitalia underdeveloped. Testes small. Hair distribution is normal. Recurrent depression. B.P. 125/70.	1946	18.3 16.2	64

normal and later rose as high as 117 units for a period. This case illustrates the possible usefulness of this estimation in excluding the presence of an adrenal deficiency in an obscure case with some of the features of Addison's disease. In case 32 the question of panhypopituitarism or Addison's disease was raised. The absence of axillary hair had apparently been present prior to the hemorrhage at labor but the history was not definite on this point. It seemed unusual for panhypopituitarism to coexist with hyperthyroidism and regrowth of a toxic goitre. The normal urinary corticoids were against a diagnosis of Addison's disease or marked panhypopituitarism. Case 33, aged 40 years (a patient of Dr. E. S. Mills), was a typical case of hypogonadism with no secondary development and small testes. He was normally grown and there was no x-ray evidence of tumor of the pituitary. The 17-ketosteroids were low but the corticoids were normal. This suggested that the pituitary was normal in so far as the adrenotropic function was concerned. Case 34 was one of marked asthenia. This asthenia had been present for years. The patient was neurotic and had a prolonged history of medical examinations with various diagnoses, among them Simmonds' disease and atypical myasthenia gravis. He was poorly developed and poorly nourished. He had been regarded as a hypogonad, his penis and testes were somewhat small but body hair growth was normal and he shaved every day. He showed no abnormal skin pigmentation and his blood pressure was 125/70. The 17-ketosteroids of 18.0 mg. and corticoids of 64 g.u. were within normal limits. These findings were against gross abnormality of the adrenal or pituitary and were thus of assistance in an obscure chronic case of asthenia with marked psychiatric features.

SUMMARY

In general then it may be said that the determination of urinary glycogenic corticoids in addition to the 17-ketosteroids is of assistance in elucidating various types of cases whose signs and symptoms suggest abnormality of adrenal function. The two determinations frequently do not parallel each other and do not give the same type of information regarding adrenal function. Both are indirect estimates of this function and are subject to various sources of error and to being influenced by factors other than adrenal cortical secretion. The biological determination of glycogenic corticoids has the advantage that it measures one type of adrenal cortical substances, those affecting carbohydrate and protein metabolism, and so far as is known, substances having this type of activity are derived only from the adrenal cortex.

It has been pointed out that there are three main types of adrenal cortical function and that it is possible that they may vary independently of one another so that the clinical signs and symptoms produced and the

metabolic findings may vary, leading to a variety of syndromes all associated with hyper or hypo function of the adrenal cortex. Physiological states, such as pregnancy and muscular exercise, are accompanied by an increased adrenal function as measured by this test, and following trauma, infection, and surgical operations, there is a rise in excretion of these substances.

It is obvious that clinical and metabolic changes ordinarily associated with variations in adrenal function may be affected by a variety of other factors. In so far as the measurement of urinary glycogenic corticoids does reflect a type of adrenal function, it may give indications of alteration in adrenal function in both physiological and pathological conditions in which other methods, such as the estimation of 17-ketosteroids or the ratio of α to β ketosteroids, do not. Thus the role of the adrenal cortex in these conditions may be further elucidated, not only in those classical clinical syndromes with which it has been associated but also in the regulation of body functions in response to exposure, to diseases other than those of the endocrine system, and to stress, which has been emphasized by Selye (9) in his work on the Adaptation syndrome.

CONCLUSIONS

The use in various types of endocrine and other clinical disorders, of the bioassay of adrenal cortical substances in urine, having the property of depositing glycogen in the liver of the adrenalectomized mouse, has been described. This has been compared with the estimation of the total neutral 17-ketosteroids.

The glycogenic corticoids and the 17-ketosteroids were in general low or not detectable in cases of Addison's disease and in panhypopituitarism with organic lesion of the pituitary.

In three cases diagnosed as anorexia nervosa the 17-ketosteroids were low but not as low as in panhypopituitarism with organic lesion. The glycogenic corticoids were normal or slightly below normal.

In four cases of active Cushing's syndrome the glycogenic corticoids were greatly increased with the 17-ketosteroids normal or slightly increased. In arrested cases the glycogenic corticoids may be within normal limits.

In four cases of simple hirsutism the 17-ketosteroids were increased and the glycogenic corticoids within normal limits.

In acromegaly in three long established cases the 17-ketosteroids and glycogenic corticoids were normal. In one recent untreated case the 17-ketosteroids were at the upper limit of normal and the urinary corticoids twice normal.

The ketosteroids and glycogenic corticoids frequently do not parallel

each other. The one may be high and the other normal or low or the reverse situation may occur.

A group of miscellaneous cases is presented indicating the usefulness of the glycogenic corticoid determination in obscure cases suspected of having adrenal dysfunction. Cases having some symptoms and signs of adrenal deficiency with normal or raised glycogenic corticoids are presented.

A general review of various methods of measuring adrenal cortical function in clinical conditions is presented and the sources of error of each is discussed.

It is suggested that the three main types of function of the adrenal: (1) electrolyte metabolism, (2) protein and carbohydrate metabolism and (3) androgenic production, may vary independently of one another both qualitatively and quantitatively, thus leading to a variety of metabolic conditions and clinical syndromes. The use of the determination of glycogenic corticoids offers a further method of estimating one type of adrenal cortical function in health and disease.

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HYPEROPHTHALMOPATHIC SYNDROME IN THYROID DISEASE

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IN RECENT years attention has been redirected to cases of thyrotoxicosis with predominance of ophthalmic manifestations. The latter include proptosis, paresis of external ocular muscles, swelling of the lids, edema of the conjunctivae and retrobulbar pain. A variety of designations has been applied to this syndrome, such as "malignant exophthalmos" and "exophthalmic ophthalmoplegia." These terms are purely descriptive and stress one or another feature of the syndrome, not all of which are present in every case. Recently two other terms have been suggested. Mulvany (12) speaks of "thyrotropic exophthalmos" a term that is objectionable on linguistic grounds since the author evidently does not wish to imply that the exophthalmos is thyrotropic but that it is caused by thyrotropic hormone. It would then be thyrotropogenic rather than thyrotropic. Means (9) speaks of "hyperophthalmopathic Graves' disease." Mulvany's term implies a very definite opinion as to the genesis of the syndrome whereas Means is deliberately noncomittal on this point but implies that the cases in question cannot be absolutely separated from "classical" Graves' disease.

The following cases illustrate certain practical therapeutic problems. Some observations made in these patients appear of basic interest and may shed some light on the pathophysiology of the exophthalmos.

Case 1. J. G., a white man aged 43, was admitted to Jefferson Hospital Jan. 21, 1944. In the summer of 1943 he developed thyrotoxic manifestations (nervousness, excessive sweating, frequency of bowel movements, insomnia, palpitations). He claims that his eyes protruded rather suddenly following an alcoholic debauch. The condition became increasingly worse and he was admitted to another hospital October 1, 1943. The BMR was at that time +85, the basal pulse rate 126. After routine preparation with potassium iodide, subtotal thyroidectomy was performed at a time when his BMR was +46 (pulse 96). He believes that his eyes improved somewhat following the operation. However, soon after December 1943 the ocular condition deteriorated considerably.

On admission, the lids of both eyes were swollen and ectropionized, exposing the entire conjuctivae. The eyeballs were bulging, the conjunctivae edematous. There were ulcerations on both corneae and there was considerable photophobia. He complained of severe "headaches" localized "behind the eyes." The eyeballs were very tender to pressure and almost entirely immobile, upward movement being impossible, downward and sideward movements considerably limited. Pressure on the eyeballs within the limits possible because of pain, showed that they could not be pushed into the orbit (Fig. 1).

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He was conspicuously slow in reacting to questions, his voice was raucous, his skin and hair dry, face and extremities showed a rather hard non-pitting swelling. His blood pressure was 120/80. The BMR was minus 21; serum cholesterol 161 mg. per cent; serum bilirubin 0.2 mg. per cent; phenolsulphonephthalein test: all dye removed. RBC 3.9 Mill. Hb. 84 per cent, WBC 6.900 with normal differential count. X-ray of the skull was negative. Urinary hormone excretion: gonadotropins non-demonstrable, estrogens less than 6 IU. per 24 hours, 17 ketosteroids 4.5 mg. equivalent of androsterone per 24 hours.

The diagnosis of severe ophthalmopathy ("malignant exophthalmos") was clear

FIG. 1. Case 1. J.G. Before treatment (January 1944).

enough. It also seemed clear that conservative measures would not become effective quickly enough to save the endangered eyes. As a first emergency measure a canthotomy and scarification of the cornea was performed and celluloid cups were placed over both eyes (by Doctor Charles E. G. Shannon, Department of Ophthalmology). Subsequently a decompressing operation (Kroenlein-Naffziger) was performed by Doctor R. Jaeger (Department of Neurosurgery), on the right side on February 4, 1944, and on the left side on March 9, 1944.

Thyroid medication was started on the day following admission. He received 0.06 gram (1 grain) daily for one week, then 0.09 gram (1½ grains) for one week, 0.12 gram (2 grains) for two weeks and 0.18 gram (3 grains) from then on. X-ray irradiation of the pituitary gland was started two days after admission, and within 10 days 900 r were applied to each field.

The clinical effect of the treatment was gratifying. The patient left the hospital on March 21 greatly improved. Treatment was continued in the Endocrine Clinic (outpatient department) with thyroid U.S.P. 0.18 gram (3 grains) daily. Figure 2 shows the result of treatment.

While the ultimate result of therapy was satisfactory the unusually slow response to thyroid medication was remarkable. On March 17 after 55 days of treatment with thyroid the BMR had hardly risen (minus 17 on March 17). By June 5, after 164 days of treatment which included one intravenous injection of thyroxin in addition to the oral medication the BMR had risen to minus 2. On June 15, fifteen mg. of crystalline thyroxin were injected intravenously. As can be seen from Figure 3 this large dose had no metabolic effect. Neither were there any other effects such as tachycardia or vasomotor phenomena.

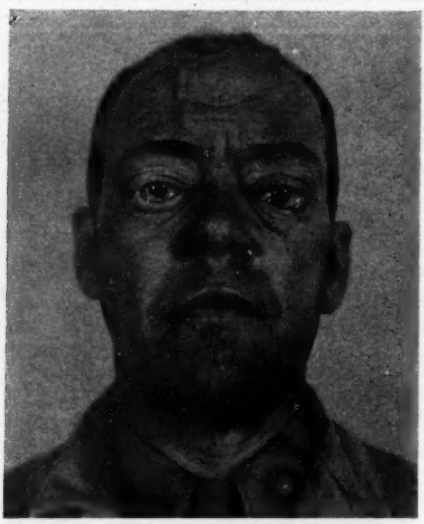

FIG. 2. Case 1. J.G., After fifteen months of treatment (April 1945).

Summary of Case 1. A 43 year old patient developed a most severe hyperophthalmopathic condition following subtotal thyroidectomy for thyrotoxicosis. From this truly n alignant ophthalmopathy he made satisfactory recovery after a decompressing operation, administration of thyroid extract, and irradiation of the pituitary gland. The patient showed a very poor metabolic response to thyroid medication, both oral and intravenous.

Case 2. C. F. a white woman aged 42, was admitted to the Endocrine Clinic on August 23, 1943 with the following history. She had been operated upon in another hospital for thyrotoxicosis two years previously. According to the report subsequently obtained from this hospital she had presented a typical picture of Graves' disease with a BMR of plus 35 and moderate exophthalmos.

The operation evidently was successful in alleviating the thyrotoxic manifestations. Her nervousness subsided, she gained weight and felt well. The condition of her eyes, however, deteriorated. The protrusion increased considerably and her lids became swollen.

On physical examination the ocular findings were very conspicuous. There was con-

siderable exophthalmos (exophthalmometer readings 23 mm. right eye, 20 mm. left eye); the lids were swollen; there was paresis of upward gaze. The bulbi were tender to palpation and attempts at ocular movements in all direction were painful. There was considerable photophobia.

She was a moderately obese woman. There were no other findings of note. Her menses were regular.

The BMR was plus 1 (basal pulse rate 70); serum cholesterol 259 mg. per cent; 17-

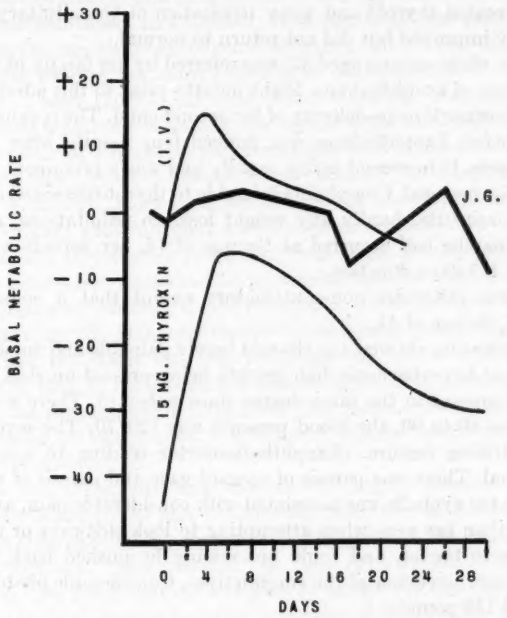

FIG. 3. Response of J.G. (Case 1) to intravenous injection of 15 mg. of crystalline thyroxin. For comparison the response of a myxedematous patient at two different levels of BMR to 10 mg. of thyroxin I.V. is given. These curves are redrawn from the paper of W. O. Thompson et al. (22).

ketosteroid excretion 7.6 mg. equivalent of androsterone per 24 hours; gonadotropin excretion varied from 0 to 42 IU per 24 hours.

Treatment was started on September 27, 1943, with thyroid U.S.P. 0.09 gram (1½ grains) daily, and after one month this was increased to 0.12 gram (2 grains) daily. After five months of treatment, by March 1944, photophobia had completely subsided, and the swelling of the lids while still present was considerably lessened. The eyeballs had visibly receded, and the exophthalmometer readings now were 17 mm. on the right eye, and 15 mm. on the left eye. The BMR, however, was unchanged, plus 1. Serum cholesterol was 171 mg. per cent. The daily dose of thyroid was now increased to 0.18 gram (3 grains), and soon thereafter to 0.24 gram (4 grains). After one year of treatment her condition was greatly improved but now appeared stationary. A course of x-ray

irradiation was given to the pituitary gland (900 r) with the hope of inducing further regression of the exophthalmos. This did not appear to have a striking result.

Treatment with oral doses of thyroid U.S.P. 0.24 gram (4 grains) daily was continued, and two more courses of irradiation to the pituitary were given. Further improvement was obtained but full normalization was not achieved.

Summary of Case 2. A woman aged 42 was operated upon elsewhere for a moderately toxic goiter. In the two years following subtotal thyroidectomy the exopthalmos increased considerably and other manifestations of ophthalmopathy developed. She was treated with desiccated thyroid and x-ray irradiation of the pituitary gland. Her condition was greatly improved but did not return to normal.

Case 3. J. C., a white woman aged 33, was referred by her family physicial to surgical service (A.) because of exophthalmos. Eight months prior to this admission she had become irritable following forceps-delivery of her second child. The pregnancy and delivery had been uneventful. Exophthalmos was noticed four months after the approximate onset of nervousness. It increased rather rapidly and was a prominent feature when she was first seen in the hospital. Complaints referable to thyrotoxicosis were mild; there was some increase in appetite, hardly any weight loss, no palpitations, and no increased perspiration. Menarche had occurred at the age of 14, her periods were regular at 27 days interval, of 4–5 days duration.

The history was otherwise non-contributory except that a cousin allegedly had thyrotoxicosis at the age of 14.

Physical examination showed the thyroid barely palpable and smooth. There was a moderate degree of hypertrichosis, hair growth being present on chin and lip (this hirsutism had been present in the same degree since puberty). There was no tremor and her pulse rate was 80 to 90, the blood pressure was 120/70. The severe exophthalmos was the most striking feature. (Exophthalmometer reading 18 mm. on both eyes.) Fundi were normal. There was paresis of upward gaze and paresis of convergence. Any attempt to move the eyeballs was associated with considerable pain, and patient turned her head rather than her eyes when attempting to look sideways or upward. On pressure the bulbi were tender, and could not readily be pushed back into the sockets. There was, however, no edema of the conjunctivae. Considerable photophobia was present. She weighed 119 pounds.

Her BMR was plus 14 (basal pulse rate 76); her serum cholesterol 234 mg. per cent; the urine analysis negative for sugar and albumen. The blood count was normal. X-ray of the skull revealed no abnormality. 17-ketosteroid excretion was 5.7 mg. equivalent of androsterone per 24 hours. Estrogen excretion was 16 MU per twenty-four hours, gonadotropins non-demonstrable.

In view of the absent, or at the very most, mild, thyrotoxic manifestations (some nervousness, slightly increased appetite) as contrasted to the marked ocular manifestations, operation was advised against and patient was transferred to the Endocrine Clinic. On August 11, 1943, treatment was started with diethylstilbestrol, 2 mg. daily. Two weeks later she stated that she felt much relaxed and generally better but there was no distinct improvement of the eyes. The daily dose was increased to 3 mg., and on September 8 to 4 mg. daily. On September 22, five and one-half weeks after treatment with diethylstilbestrol had been started the exophthalmos was clearly receding and exophthalmometric measurements now were 12 mm. on the left and 13 mm. on the right eye. The rather large doses of diethylstilbestrol were tolerated without any discomfort or toxic manifestations. Stilbestrol apparently failed to suppress pituitary gonadotropin production. An assay performed at this time showed an excretion of 16 IU per twenty-

four hours. On October 15, 1943, the daily dose was increased to 7 mg. About this time she noticed further and considerable improvement now being able to "turn her eyes rather than to have to turn her head." Her eyes felt "relaxed." She had gained five pounds (125½) and felt completely well. A BMR was determined at this time as a routine checkup, and a reading of plus 52 was recorded, with a basal pulse rate of 80. The serum cholesterol was 146 mg. per cent. The BMR determination was repeated two weeks later with identical result (plus 53, pulse 96). Because the patient felt entirely well and had no thyrotoxic manifestations, and because the ocular signs had been greatly improved, the medication was continued. In February, 1944, her BMR was plus 56 (pulse 84); her serum cholesterol 140 mg. per cent. Glucose tolerance test showed the following values: fasting blood sugar 109 mg. per cent, ½ hour after oral ingestion of 100 grams, glucose 167 mg. per cent, 1 hour 156 mg. per cent, 2 hours 122 mg. per cent. The patient had now had high basal metabolic rates and low serum cholesterol values for four months but again the absence of thyrotoxic manifestations both subjective and objective was noted. The weight had further increased to 129 pounds. The estrogen excretion was at this time found to be high (more than 100 MU per 24 hours). The serum estrogen was 60 MU/100 cc. Urinary gonadotropins were not demonstrable.

It was now decided to withdraw stilbestrol medication in order to evaluate a possible influence of the estrogenic compound in producing this unusual picture. In order to avoid massive withdrawal bleeding the withdrawal of the drug was carried out gradually beginning on February 25, 1944, and finally discontinued entirely on March 17, 1944. On March 31, 1944, the BMR was plus 53 (pulse 90). Thyrotoxic manifestations remained absent. On May 12 her BMR was plus 22 (pulse 80), her serum cholesterol 146 mg. per cent. In the next several months the BMR varied between plus 20 and plus 30. Her weight increased to 136 pounds. She felt well and the improvement of the ocular manifestations was maintained.

In April, 1945, as a therapeutic test, she received Lugol's solution, 15 drops daily for fourteen days. Her BMR fell to plus 7. Clinically there was no change, and when questioned she stated that she had felt so perfectly well prior to the iodine medication that she could not report any "improvement." In June 1945, six weeks after discontinuing iodine medication, the BMR had risen to plus 31 (pulse 84). In spite of the absence of clinical manifestations of thyrotoxicosis it was deemed inadvisable to wait for such to appear. Treatment with thiouracil was started on June 18, 1945. At first the drug was well tolerated but on August 8 she was admitted to this hospital with a very severe agranulocytosis. The drug was immediately withdrawn and treatment with penicillin and large doses of Vitamin B (Brewers Yeast) given. She recovered completely.

Since that time her condition has remained stationary. She has had two more courses of x-ray treatment to her pituitary gland (600–900 r. each). Her weight is constant, she feels well but still has a moderately elevated BMR (plus 20).

Summary of Case 3. A woman aged 33 years was observed with very mild (border line) thyrotoxic manifestations and considerable ophthalmopathy. Treatment with large doses of diethylstilbestrol resulted in marked improvement of the ophthalmopathy. During the period of treatment with the estrogenic compound, at a time when her eyes were considerably improved, her BMR rose and the serum cholesterol decreased but there were no clinical manifestations of thyrotoxicosis. Diethylstilbestrol was withdrawn. The BMR decreased without, however, reaching normal levels, and the serum cholesterol remained low. A therapeutic-diagnostic test with iodine medication resulted in prompt fall of the BMR to normal values, with a subsequent rise after discontinuing iodine medication.

Case 4. E. C., a white man aged 49, in October 1943 first noticed double vision and difficulty in gazing upward. The latter was limited to the left eye. At about the same time he became very nervous, perspired excessively and developed tremor. His appetite was poor, and he lost 20 pounds in a few months. He was seen by an internist whose studies revealed the following: BMR plus 30 (basal pulse rate 96); serum cholesterol 104 mg. per cent; X-ray of the skull negative; pulse rate 100 to 120; Wassermann and Kahn reaction in the serum negative. Spinal fluid examination was normal. There was marked tremor of the hands. Both eyes showed limitation of outward rotation, slight limitation of inward movement and marked impairment of upward gaze bilaterally, but more marked on the left. Myasthenia gravis was considered but there was no response to prostigmin. Thiouracil treatment was started but had to be discontinued after 10 days because of drug fever and rash.

During the following year the patient was under the care of his family physician, and received symptomatic treatment only (sedatives). He was then referred to the surgical service of Doctor T. A. Shallow for thyroidectomy. Upon admission his condition was the same as described above; his ophthalmopathy had neither improved nor deteriorated, he still had tachycardia (120), his BMR was now plus 17 (basal pulse rate 98).

Operation was advised against. The thyrotoxicosis, while mild, caused enough discomfort to warrant treatment and was brought under control by cautious x-ray irradiation to his thyroid gland. At the same time deep x-ray therapy to the pituitary gland was started. He has so far received three courses, 900 r each, with no change in the ocular condition.

Summary of Case 4. A man aged 49, developed mild thyrotoxicosis and severe ophthalmoplegia with exophthalmos at the same time. The mild thyrotoxicosis was easily controlled by x-ray irradiation of the thyroid gland. Deep x-ray therapy to the pituitary gland was employed. There has so far been no improvement of the opthalmopathy.

Case 5: S. H., a white man aged 38, presented himself with the following history. Two years previously he had noticed bulging of the eyes. At the same time he began tiring very easily, and was drowsy most of the time. His skin became dry and perspiration ceased almost entirely. The eyes became very painful, the lids and the conjunctivae became swollen, and an ulcer of the cornea developed. He was seen elsewhere, and a BMR of minus 40 was found. He received thyroid medication and x-ray irradiation of the pituitary gland. His condition improved considerably.

When seen by us two years after the onset he was still taking desiccated thyroid tablets. He had moderate exophthalmos, but no swelling of lids or conjunctivae. Ocular movements appeared entirely free. His skin was moist and he was alert. He was advised to continue treatment under the care and supervision of the physician who had been treating him.

Summary of Case 5. A man aged 38 developed a severe ophthalmopathy with exophthalmos, swelling of lids and conjunctivae and ulceration of the cornea simultaneously with the onset of spontaneous myxedema. There was no indication from the history that the myxedema had been preceded by thyrotoxicosis. Treatment with desiccated thyroid and x-ray of the pituitary gland resulted in almost complete recovery, with only a slight residual exophthalmos.

DISCUSSION

The problem of "hyperophthalmopathic Graves' disease" has recently been reviewed by Means (9, 10). Briefly there is much evidence that exophthalmos in thyrotoxicosis is not caused by excessive amounts of circulating

thyroid hormone. In animals all manifestations of thyrotoxicosis except exophthalmos can be produced with thyroid hormone or with thyroxin. Exophthalmos, however, can be produced by administration of pituitary extract containing thyrotropic hormone and the exophthalmos is more readily induced and more severe in thyroidectomized animals receiving pituitary extracts (4, 5, 8, 19). In humans the exophthalmos of Graves' disease increases in more than 50 per cent of all cases following thyroidectomy as was shown by actual measurements of the exophthalmos (2, 21). The fallacious impression of improvement of exophthalmos in such cases is due to the "stare" being diminished when thyrotoxicosis is under control.

The conclusion from these observations is that the exophthalmos is caused by a pituitary factor, either the thyrotropic hormone or some other factor as yet unknown. Release of the anterior pituitary following thyroidectomy ("taking the brakes off") leads to an increased secretion of thyrotropic hormone as has been shown by assay of thyrotropic hormone in the urine (6), and thereby causes increase in exophthalmos.

The pathological changes in the orbital tissues (fat, muscle) have been extensively studied in humans (12, 13, 18) and experimental animals (18). Edema, swelling of the extrinsic muscles. and increase of the retrobulbar fat are the main findings.

There still is some difference of opinion as to whether this mechanism holds true for all types of exophthalmos. Mulvany (12) has proposed a dualistic theory contrasting the exophthalmos in ordinary Graves' disease ("thyrotoxic exophthalmos") with that in cases without thyrotoxicosis ("thyrotropic exophthalmos"). The former, the thyrotoxic exophthalmos, Mulvany explains by action of the thyroid hormone on the smooth muscles of the orbit as well as on the extraocular muscles, reviving in somewhat modified form the old theories attributing exophthalmos to the sympathicomimetic action of thyroid hormone.

On the other hand, Means (9, 10) and others strongly advocate a unitarian theory of exophthalmos. There are all transitions from "classical" Graves' disease with severe thyrotoxicosis and exophthalmos through euthyroid to hypothyroid and myxedematous conditions with severe ophthalmopathy. Nor is the "type" of exophthalmos constant or fixed in any one patient, and what started out as "classical" Graves' disease may later become "hyperophthalmopathic."

Mulvany has based his dualistic theory largely upon morphological studies of the intraorbital tissues. Rundle and Wilson (18) on the basis of histological studies of a similar material find evidence of a unitarian genesis of all exophthalmos, "classical" and "hyperophthalmopathic." It is per-

haps possible that what Mulvany interprets as two different processes may be different stages and different degrees of the same condition.

The cases presented in this paper fully support the theory that the exophthalmos is not the direct result of the hyperthyroidism, viz., of the excess of circulating thyroid hormone. They do not disprove the existence of a second type (thyrotoxic) exophthalmos, but we find the evidence for such occurrence unconvincing in the light of facts briefly mentioned above and extensively discussed by Means (9, 10). In two of the cases (No. 1, 2) the ophthalmopathy became much more severe following subtotal thyroidectomy, and in one (Case 5) the ophthalmopathy developed simultaneously with the onset of myxedema, which apparently had not been preceded by thyrotoxicosis, and was, therefore, not the result of a "burnt out" toxic goiter.

Case 3 of our series is particularly instructive. This patient's disease started with severe ophthalmic manifestations (exophthalmos, paresis of extrinsic muscles, but no swelling of lids) with almost no hyperthyroidism. The BMR rose, and the serum cholesterol decreased at a time when the ophthalmopathy was considerably improved under treatment. This case warrants further discussion from another standpoint. The question presented itself as to whether prolonged administration of large doses of diethylstilbestrol had caused the rise in BMR as has been described in the rat (7). On the other hand the claim has been made that estrogenic hormone decreases thyroid function and attempts have even been made to use estrogens in the treatment of thyrotoxicosis (3). However, we have not been able to demonstrate an inhibitory effect of estrogen upon the uptake of radioactive iodine by the thyroid (14). The fact that the BMR remained elevated after cessation of stilbestrol treatment though on a lower level than was present during administration of the estrogen, and responded promptly and typically to iodine medication makes us believe that this patient went into spontaneous exacerbation of thyrotoxicosis. This exacerbation was in all probability independent of any therapy. The unusual feature presented by this patient, namely hyperthyroidism (as evidenced by consistently high BMR and low serum cholesterol and typical response to iodine administration) in the absence of any clinical manifestations is difficult to explain on the basis of present knowledge.

Metabolic response to thyroid and thyroxin

Case 1 showed a surprisingly slow and inadequate response to orally administered thyroid extract. It is known (21) that the metabolic response to thyroid administration depends on the level of the BMR, and not on the presence or absence of the thyroid gland. Evidently there must be ex-

trathyroidal factors of utilization or destruction of the administered hormone as has been recently discussed by Danowski et al. (1).

In order to exclude the uncontrollable factor of absorption of orally administered thyroid material, it was decided to study the reaction to intravenous injection of crystalline thyroxin. This was carried out at a time when the BMR had finally risen to between 0 and −2. As can be seen from Figure 3 there was no rise of BMR after the thyroxin injection. For comparison the response to a smaller intravenous dose of thyroxin (10 mg.) at two levels of BMR is charted in this figure, the latter two curves taken from Thompson's paper (22). It must be concluded from these observations that either in this case the oxidative processes were peculiarly insensitive to thyroxin, or that the injected thyroxin was inactivated or destroyed by some antagonistic agent. No definite answer is possible at this time to the question raised by the observations. A peculiar insensitivity to administered thyroid occurs in some humans (17) and apparently quite regularly in the dog (1). Rawson et al. have shown that thyrotropic hormone is inactivated *in vitro* by thyroid tissue (15). There is no information as to whether this inactivation is reciprocal, as might be the case if the thyrotropic hormone would combine in some way with the thyroid hormone. Should this hypothesis be verified it would explain the poor reaction to thyroxin in a condition in which an excessive thyrotropin secretion is supposed to exist. Case 2 revealed a similar behavior towards administration of thyroid material, but it was less marked than in case 1.

Prevention and Treatment

Our cases illustrate the importance of avoiding thyroidectomy in potential cases of severe ophthalmopathy. This has been stressed by a number of authors but is unfortunately not generally recognized in practice. The decision against operation is easy in cases of ophthalmopathy with normal thyroid function, or with hypothyroidism (Case 5), or with a mild borderline thyrotoxicosis (Cases 3 and 4) contrasting with the severe ophthalmopathy which seems "out of proportion." The decision may not be simple in cases in which severe ophthalmopathy is associated with severe thyrotoxic manifestations. This was probably the situation presented by our cases 1 and 2, both of whom we saw only after operation had been performed elsewhere. There may not be any simple and unequivocal warning sign in such cases. It has been suggested that an unusually low BMR resulting from iodine medication is a danger sign. Whereas the BMR under medication with iodine usually levels off at more or less hyperthyroid levels, and only in mild cases is normalized, there are cases in which values as low as minus 20 have been observed, and these have been considered as

potential "malignant exophthalmos" cases (5). We are unable to judge our two postoperative cases from this angle because, according to the reports received from the respective hospitals, no attempt had been made preoperatively to determine the lowest BMR obtainable, or in other words to wait for the "leveling off" which is the only reliable indicator of the maximal iodine effect obtainable in each case. However, there are cases on record of severe ophthalmopathy developing following thyroidectomy in which the BMR did not drop to unusually low values (11).

Prevention of this condition depends on judicious evaluation of each case. Severe ophthalmopathy out of proportion to the thyrotoxic manifestations is a clear contraindication to thyroidectomy. Great care must be exercised in cases in which the disproportion is not so evident because of severe thyrotoxicosis. The effect of iodine should be studied carefully, and medication continued to the lowest obtainable BMR value. In case of the slightest doubt, operation should be decided against. This is particularly true in view of the availability of other methods of treatment of Graves' disease. A discussion of the relative merits of x-ray therapy, therapy with antithyroid drugs (thiouracil, etc.), or perhaps with radioactive iodine, are beyond the scope of this paper. These methods of treatment appear to be less dangerous to the eyes perhaps because the suppression of thyroid function is more gradual than that accomplished by operation. The antithyroid drugs would seem to offer the additional advantage of complete reversibility of the antithyroid effect, permitting one to set the thyroid function at any desired and tolerated level.

The emergency treatment of the ophthalmopathy is that of protection of the eyes by local measures, followed by decompressing operations (see case 1). The rationale for this operative treatment is the recognition of edema, and increased ("hypertrophic") fat of the retrobulbar structures as the anatomical basis of the ophthalmopathy. Medical treatment, alone in cases not presenting immediate danger, or following surgical emergency measures, is directed towards suppressing excessive secretion of the anterior lobe of the pituitary gland. Large doses of estrogenic hormones, both natural and synthetic, are known to suppress pituitary function; at lower dosage levels, specifically the follicle-stimulating hormone secretion, in larger doses over longer periods of time, the secretion of other pituitary hormones also is inhibited. Suppression of the thyrotropic hormone by this "shotgun action" (Salter) is attempted in treating the ophthalmopathy with estrogens. The effect of thyroid medication on the pituitary gland is primarily that of suppression of the thyrotropic hormone. In the light of recent experiments, thyroid hormone may perhaps also act by inactivating (neutralizing) thyrotropic hormone peripherally (15). Thyroid medication

would then be the procedure of choice at least in the euthyroid or hypothyroid cases (our cases 1, 2, 5). Whether it can be safely given over long periods of time, and in sufficiently high doses in cases of thyrotoxicosis is as yet uncertain. Rienhoff (16) prepared thyrotoxic patients for operation successfully with desiccated thyroid, producing involution of the gland similar to that obtained with iodine. The patients tolerated thyroid medication well. We have thus far decided not to use thyroid medication in the two thyrotoxic cases. X-ray therapy of the pituitary gland, of course, attempts direct suppression of the overactive gland. It is used very commonly but almost always in conjunction with one of the two hormonal methods outlined above. In the one case in which we have used it alone the result was not satisfactory. From a theoretical standpoint one might question the radiosensitivity of nontumorous, albeit hyperfunctioning pituitary tissue.

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VIRILIZING OVARIAN TUMORS*

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THE purpose of the present paper is to describe the clinical, histological and hormonal findings in a case of a diffuse arrhenoblastoma, one which, in accordance with ideas hitherto, will be regarded as an "adrenal-like" ovarian tumor; furthermore, to examine whether it is possible, from the cases in the literature, to find transitions in the symptomology between typical arrhenoblastomata and "adrenal-like" ovarian tumors, thus forming a parallel to Teilum's histological studies. A summary will then be given of the available hormone studies, and mention will be made of certain characteristic findings in cases of virilizing ovarian tumors.

Virilizing ovarian tumors are rather uncommon, but very interesting because they are functioning endocrine tumors. There is a great diversity of opinion on the histogenesis and classification of these tumors; little is known of their hormonal relations, which seem to embody a number of problems.

Usually a distinction is made between two different virilizing ovarian tumors: arrhenoblastoma and "adrenal-like" ovarian tumor.

The arrhenoblastomata received their name from R. Meyer (17) in 1930. His great merit was that he was able to show the connection between types which histologically were far apart and thereby put them together into one form of tumor. He divided the arrhenoblastomata into three groups according to the degree of differentiation. He considered the testicular adenoma (adenoma tubulare), which was first described by Pick (21) in 1905, as the most differentiated. An intermediate group comprises histologically both tubular and diffuse areas. This forms the transition to the third group, the least differentiated form, which has a diffuse, "sarcoma-like" structure. The two latter groups sometimes contain more or less numerous epitheloid, often lipoid-containing cells. Owing to their greatly varying appearance it is sometimes difficult to make a purely morphological division from feminizing tumors of the granulosa cell group (diffuse type). The hormonal effect is greatest in the diffuse forms and is rarely observed in the purely tubular adenoma. We have no definite knowledge of the hormone-producing cells.

About sixty cases of arrhenoblastoma have been described. "Adrenal-like" tumors are still more uncommon. (The one described below is No. 19.) They consist of large, light-colored, often lipoid-containing, epitheloid cells. There is no tubular structure to be seen, for which reason the tumor as-

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sumes a uniformly diffuse appearance. Hitherto the form has proved to be virilizing. The nature and genesis of these tumors have given rise to considerable disagreement, for which reason they are known by many different names: adrenal-cell tumors, adrenal cell rest tumors, hypernephroma in the ovary, luteoma, masculinovoblastoma, etc. Morphologically the cells are very similar to both lutein cells and cells in the adrenal cortex.

One aspect of the disagreement has been as to whether the tumor comes from aberrant adrenal cortex or is a luteinized granulosa cell tumor or a true lutein cell tumor. Schiller (24), e.g., rejects the theory of luteinized granulosa cell tumors, also for morphological reasons. He admits only two possibilities: they are either tumors of adrenal tissue or tumors of a form arising out of a one-sided development of interstitial cells corresponding to Leydig cells in the testicles. As stated, such epitheloid cells occur in larger or smaller numbers in typical arrhenoblastomata too. One-sided development of mesenchymal cells is comprehensible according to Fischel's theory (6), which in the testicles traces all epithelial elements in the ducts, except spermatogonia, from a common mesenchyme nucleus.

Schiller arrives at the conclusion that the question is one of aberrant adrenal cortex, as he is unable to find the histological transition between arrhenoblastomata of ordinary type and "luteomata." The commonest opinion now is that they are tumors emanating from adrenal cortex.

With his studies of homologous tumors in ovary and testis, Teilum (28–30) has thrown entirely new light upon these problems. He has succeeded in demonstrating tumors in testicles (called androblastoma series) showing total morphological congruence with ovarian arrhenoblastomata in all the various forms of differentiation, and also in demonstrating gradual transitional phases between these forms. In addition, by demonstrating transitional phases in typical arrhenoblastomata to parts with uniform (diffuse, alveolar or tubular) structure—corresponding to the structure of virilizing ovarian tumors which hitherto have been regarded as "adrenal-like"—Teilum has extended the arrhenoblastoma series so as to comprise these diffuse types as well. The material seems convincing. It may be taken as an affirmation of R. Meyer's doctrine regarding ovarian arrhenoblastomata, that these tumors all emanate from a special, testicular anlage. It means furthermore that "adrenal-like" ovarian tumors are most naturally to be regarded as a variant of a diffuse arrhenoblastoma, some of which show differentiation in the direction of "interstitial cell tumors" in the testes, and that the idea of adrenal tumors in the ovaries may be dropped.

We may imagine the entire series of androblastomata as tumors growing from a mutually potential male mesenchyme nucleus with differentiation in various directions (tubules, interstitial cells) and with various degrees

of differentiation. As regards their direction of differentiation, testicular adenomata and "adrenal-like" ovarian tumors may be regarded as polar opposites within the androblastoma series.

Among the diffuse forms we may possibly take it for granted that the tumors are differentiated in various directions. Among "adrenal-like" tumors the majority contain lipoids. Possibly these signify a differentiation in a direction other than that taken by the few non-lipoid containing, "adrenal-like" tumors, so that those containing lipoids need not necessarily be considered as having evolved out of a continued development of those not containing lipoids.

Kepler and co-workers (11) consider, on the basis of 14 cases of "adrenal-like" ovarian tumors (thirteen from the literature and one of their own), that clinically it is possible to distinguish between arrhenoblastomata and "adrenal-like" tumors. Apart from virilism, the latter are associated with many of the signs characterizing Cushing's syndrome (hypertension, hyperglobulia, striae caeruleae, diabetes), whereas the arrhenoblastomata cause pure virilism. They leave open the question of how the tumors are to be regarded histogenetically.

CASE RECORD

S.M.H., an unmarried woman, 50 years old, was transferred here from the Neurological Department of the Rigshospital with the diagnosis: endocrine affection; meningoencephalitis hypothalamus region.

There is no record of endocrine affection in the family. The parents were not related.

Menses from 16th year were always regular, lasting three or four days every fourth week. Menstruation ceased at 44th year. At the same time she began to lose her hair and in less than a year was almost completely bald; has since worn a wig. When she was 46–47 years old a vigorous hair growth began to appear on her chin, cheeks, chest, abdomen and back; the baldness persisted. Shortly afterwards there was a sudden increase of weight, about 5 kg. (11 lb.) in a month. At the same time the skin of her face, chest and back became very greasy with large pores. She had always had slight acne, but this did not spread. She was afflicted with strong and protracted flushes, during which her face and neck turned bluish-red and her head felt as if it would burst. The attacks lasted about two hours. During the last two years she had noticed a growth of the clitoris, but no change in the breasts. At about the age of 45 her voice became husky, unclear, and later it "broke" like that of boy at pubescence. As she had to talk a great deal in her occupation, the voice change was a considerable embarrassment and she had to take lessons in voice control.

The patient was of a diffident nature and shy of making contact with people. From her 44th year she withdrew more into herself, became depressed, strangly split, felt tired, got out of balance. From previously being on the best of terms with others, she became hot-tempered, excitable, abusive and quarreled with people. There came a change in erotic attraction; she began to take notice of young girls and their appearance.

During the four years preceding admittance to the hospital she had been given energetic treatment. For about a year she was given a course of di-iodothyrosine tablets, as her doctor thought that her baldness was due to increased metabolism. After her sudden

increase in weight—which set in a month after she had ceased taking the di-iodothyrosine tablets—she was treated with tablets of glandular thyreoidine, 1 tablet three times daily for three years. In the same period she was periodically treated with estradiol-monobenzoate. She had diathermy treatments for the hypertrichosis but without success.

Four or five months prior to coming to the Steno Memorial Hospital she was run over. She sustained a blow in the back, but no head trauma. Thereafter she suffered from dizziness and her memory began to fail. She had no headache. On account of her vertigo she was admitted to the Neurological Department of the Rigshopsital in November, 1944.

Here nothing definitely abnormal was found neurologically, and she was transferred to the Steno Memorial Hospital for further observation for endocrine disease.

FIG. 1 FIG. 2 FIG. 3

Patient with virilism, before operation.

We found the following: weight and height normal; appearance strongly virilized; no head hair except at temples and nape (her baldness exactly like seborrhoic baldness in a man); very vigorous hair growth on cheeks, lips, chin, neck, back, chest, abdomen (especially in the median line), lower legs and forearms; a less vigorous growth on thighs and upper arms. The hairs everywhere were long, rather thin and soft.

The skin was very seborrhoic and large-pored, especially on the face, but also on the other sites of seborrhoea. The skin on the face and neck was dark red, as if it were more weatherbeaten than elsewhere. There were no acne pimples, ecchymosis or perniosis of the skin; no striae caeruleae. Her muscular relief was prominent, especially on the shoulders, upper arms and thighs. The subcutaneous veins of the forearms were very conspicuous. Her breasts seemed quite normal for her age. Her voice was dark, husky, but not particularly virile (voice production courses!). The patient was restrained, depressed, touchy.

Gynecological examination by two different gynecologists at an interval of a month showed: labia majora natural; clitoris considerably enlarged almost to the size of the end of a man's middle finger; hymen adest, speculum could not be introduced; uterus of nor-

mal size, freely motile. At the left lateral margin, almost up at the tuba corner, an intumescence (fibroma) the size of a large nut could be felt. Nothing definite could be felt at the sides of the uterus. Suggested: exploration under narcosis, the exploration find not being quite certain.

The remaining objective, routine examination of the organs revealed nothing abnormal; no tenderness or swelling could be found in the adrenal regions or the abdomen.

The photographs (figs. 1, 2 and 3) show some of the features mentioned. If the medical history were not known, the photographs might be mistaken for those of a man with gynecomasty. Bingel's expression (1) "male potator" is very apt.

Other examinations of the patient[1] showed: height: 162 cm. (64 in.); weight: 58 kg. (128 lb.); blood pressure: 110/60–120/70; urine: no albumin, no sugar.

Blood: W.R. neg. S.R. 4 mm., Hgbl. 110–115 per cent, red corpuscles 5.23 millions, white 5880; differential count of white corpuscles normal. Red blood picture natural. Vol. index 1.07, color index 1.10. Serum calcium 9.6–9.9 mg. per cent. Total base: 145.8 m.eq/litre. Serum sodium (1) 149 m.eq/litre, (2) 134 m.eq/litre; serum potassium (1) 5.0 and (2) 5.8 m.eq/litre.

The fasting blood sugar was determined thirty-two times; nine times it was over 100 mg. per cent, once it was 122 mg. per cent. On two different occasions glucose tolerance curves with 70 Gm. glucose took the same course: an increase from 90 to 220 mg. per cent in an hour; the initial value was reached after 2¼ hours. Urine sugar tests 1, 2, 2½ and in one test 3½ hours, after commencing the glucose load, gave +sugar.

Standard metabolism: +33, +14, +17, and +12 per cent of normal.

Spinal fluid: Clear; cells 0/3; glubulin 1–(2?), albumin 26–27, Pandy +, W.R. −.

Eye examination: Limits of visionary field natural; no color-hemianopia; pupil reactions and eye movements natural; ophthalmoscopy natural, papillae especially being natural.

X-ray: Skull: Marked diffuse symmetrical thickening and sclerosis of squama frontalis (hyperostosis cranialis interna). At the thickest spot the cranial wall measures 14 mm.; sella turcica natural.

Spine: No osteoporosis.

Urinary organs before and after injection of Uroselectan B: nothing abnormal, particularly in the adrenal regions.

TABLE 1. THE EXCRETION OF ANDROGEN, 17-KETOSTEROID, ESTROGEN AND GONADOTROPIN IN 24-HOUR URINE BEFORE AND AFTER OPERATION FOR VIRILIZING OVARIAN TUMOR

	Dec. 16, '44	Jan. 5, '45	Jan. 7, '45	Feb. 1, '45	Feb. 15, '45	Mar. 6, '45	Mar. 14, '45	Aug. 24, '45*	Jan. 7, '46
Gonadotropin (m.u.)	<50			<50			<50	>50	
Estrogen (m.u.)	>20 <200			<20			<20	ca. 40	
Androgen (i.u.)	50	65	15		3	8	3	6	3
17-Ketosteroid (mg)	12		17	1.8	3.2	0.1	4.0	8.6	

* Treated with stilbestrol 0.1 mg. ×3 daily from Apr. 7 to Aug. 21, 1945.

[1] The examinations were made partly at the Rigshospital, partly at the Steno Memorial Hospital.

HORMONE TESTS OF URINE

All the hormone tests made before and after the operation are shown in Table 1.

The androgen was determined by the cock's comb method with direct application after Fussgänger subsequent to simultaneous hydrolysis and benzole extraction of the urine and dissolving of the evaporated extract in oil. Comparison with dose-response curves. (See Hamburger, et al., 1945 (9)). The determinations were carried out at the Hormone Department (Chief: C. Hamburger, M.D.), State Serum Institute.

The 17-ketosteroids were determined by Callow's method (4). The analyses were made at the Biological Laboratory (Chief: K. Pedersen-Bjergaard, Ph.D.), Leo Chemical Works.

DIAGNOSTIC CONSIDERATIONS

Judging from the clinical indications, the patient probably has a benign tumor or hyperplasia in an endocrine gland, which presumably is either the hypophysis, the adrenal cortex, or the ovary. Consideration must also be given to the possibility of an affection of the brain in the vicinity of the hypophysis (the spinal fluid analysis). As all the cardinal symptoms of Cushing's disease and of Cushing's syndrome (adiposity, hypertension, striae caeruleae, osteoporosis) are absent, and there are merely a slightly reduced carbohydrate tolerance and hyperostosis cranialis interna as the sole—very diffuse and indefinite—signs of a lesion of the hypophysis, it is improbable that there is a primary affection of the hypophysis. Partly for the same reasons a cerebral lesion is unlikely. The spinal fluid analysis is hard to evaluate, being so isolated with no other abnormal neurological find. It might rather be associated with a possible encephalitis in conjunction with the severe influenza she had when 24 years old; this would then be the only remaining sign of that illness.

As the virilism is almost pure, the differential diagnosis must be between an ovarian tumor and an adrenal cortex tumor, or hyperplasia. Gynecological examination failed to reveal any ovarian tumor. X-ray examination showed no enlargement of the suprarenals. (Perirenal air insufflation was not employed, as it is considered dangerous.) The serum sodium and potassium analyses were normal at the first examination, whereas at the second one sodium was slightly low and potassium somewhat high. This is a change in the same direction as that seen in Addison's disease and opposite to what has been observed in the case of some adrenal cortex tumors. The total base is also low.

Hormone tests: An excretion of 65 i.u. of androgen and 17 mg. of ketosteroid in 24 hours at the age of 50 must, after the author's investigations (19) and experience, be described as high for this age, though not necessarily pathologically high. It tells us that the patient may possibly have a functioning tumor, but not where it is situated. To be of diagnostic importance the output of hormone metabolites must be very large. Up to the present, at any rate, they have been found only in cases of adrenal cortex tumors. On the other hand, the fact that hormonal values are normal or only slightly increased does not exclude an adrenal cortex tumor. In the present case the hormonal values agree well with an ovarian tumor, but the investigations here are few as yet. (See later.)

Treatment

It was not possible to make a fully exact diagnosis prior to the operation. As there was nothing in the clinical picture as a whole that argued against a virilizing ovarian tumor, the conclusion was that in all probability the patient had a benign ovarian tumor but that a lesion of the adrenal cortex could not be excluded.

Laparotomy was therefore decided upon, with an examination of the ovaries and palpation of the suprarenals from the peritoneal cavity.

Laparotomy was performed on the 24th of January, 1945 by Dr. H. Wulff. He removed a smooth tumor the size of a hen's egg from the right ovary and a fibroma of about the same size from the left margin of the uterus. Digital exploration of the suprarenals from the abdomen showed nothing abnormal, and all other organs were found to be normal.

Description of preparations: The right ovary had been transformed into a tumor measuring 4.5 × 3.5 × 2 cm., weight 22 Gm. The surface was grayish-white, quite smooth, and covered with a thick tunica albuginea. Inside this was a brown-red, somewhat moist tissue. Microscopically there was peripherally a narrow or somewhat broader border of a cellular ovarian stroma with small, slender cells. A few fibrous bodies were embedded in this stroma. Within this stroma zone was a node consisting of narrow or broader trabeculae of closely packed roundish or polygonal, medium sized or bigger cells (fig. 4). The cytoplasm was fine grained, fairly abundant, and in places vacuolized. The nuclei were round, sharply contoured, varying somewhat in size, and with an extremely slender chromatin structure. The nucleoli were often distinct. There were no mitoses. The cell trabeculae were spun with an extremely fine-threaded, richly capillarized, rather hyperemic stroma. An examination of the numerous sections reveals no glandular arrangement of the cells. Fat staining was negative. There was no sign of malignancy.

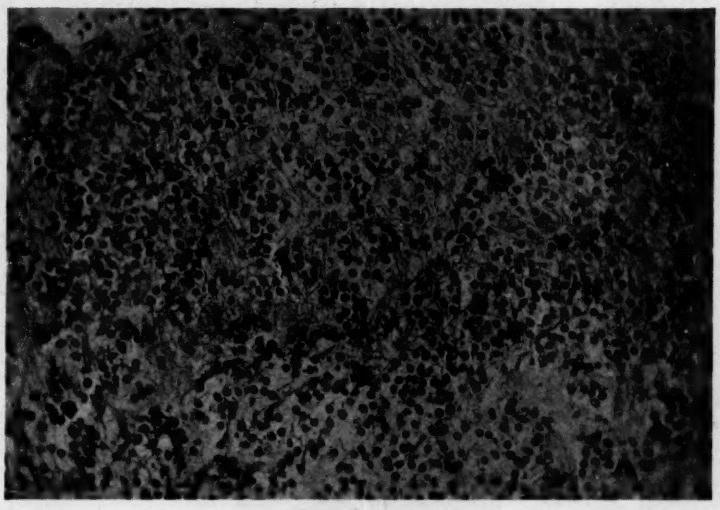

FIG. 4. Section of ovarian tumor: diffuse, no lipoids demonstrable. Arrhenoblastoma diffusum.

Thus the histological structure of the tumor agrees with what has previously been regarded as an "adrenal-like" ovarian tumor of the diffuse, non-lipoid containing type. Dr. Teilum, who has been good enough to make a close examination of the histological preparations, considers, after comparison with other gonadal tumors, that this must be a purely diffuse tumor of the androblastoma series with differentiation in the direction of testicular interstitial cell tumor (30).

Ovarian tissue from the left ovary consisted of a cellular stroma with slender cells. In the stroma were a few fibrous bodies and small follicle cysts. There were no inflam-

matory infiltrates. The right tube was microscopically normal. The fibroma showed nothing unusual under the microscope.

Follow-up examination: The post-operative course was uncomplicated. The patient was discharged on March 17, 1945, from the Steno Memorial Hospital. Virilism was even then disappearing fast. Menstruation had not returned.

Seven months after the operation the patient was admitted again for control examination. She stated that ten weeks after the operation she had had severe flushes, for which her doctor treated her with stilbestrol benzoate 0.1 mg. three times daily, with good effect on the flushes. After ten weeks of this treatment she had vaginal bleeding for

FIG. 5 FIG. 6

Patient 7 months after removal of ovarian tumor.

eight days. This had not recurred. Together with the bleeding there were slight pains in the left fossa iliaca, and they had since recurred now and then.

Objective examination (figs. 5 and 6): The patient was still psychasthenic, depressed, restrained, absorbed in her earlier affection. Looked climacteric. She repeated that she had been relieved of great pressure and inner tension.

About her appearance there was now nothing remarkable, but she was slightly aged. She had a feminine effect. The hypertrichosis had gone completely. Beyond the classical normal hair there was only a slight covering on legs, forearms and hands. On her head the hair had grown somewhat but was still very sparse. The skin of the scalp was natural. The seborrhoea had disappeared. The physical contours were more rounded, feminine; the breasts a little more full than before. Patient's weight was unchanged. Speech

and voice were feminine and light. The clitoris was still slightly enlarged, but was much smaller than before treatment.

Other examinations: Weight: 58 kg. (128 lb.); height: 162 cm. (64 in.); blood pressure: 110/75; urine: albumin, sugar.

Blood: S.R. 4 mm., Hgbl.: 100, red blood corpuscles 4.5 millions, white 6360. Serum cholesterol 172 mg. per cent.

Fasting blood sugar determined 20 times varied between 88 and 122 mg. per cent; on nine occasions the value was over 100 mg. per cent, once over 120 mg. per cent. Glucose tolerance curves with 70 Gm. glucose showed an increase from 97 to 220 mg. per cent in 45 minutes. The initial value reached in 2 hours 25 minutes. +S in urine an hour after beginning of load −S 2 and 2½ hours after.

Standard metabolism: 90 and 102 per cent. Hormone excretion: See Table 1.

Spinal fluid: 0/3 cells, albumin >20 <30, globulin >1 <2.

Gynecological examination: Hair now natural. External genitals natural except clitoris, which was as large as half of the end of a little finger, but judged to be only half as large as at last examination. Inspection showed nothing out of the ordinary. Recto-vaginal exploration found the uterus natural; nothing abnormal felt in right side. Left ovary felt about the size of a green walnut, somewhat tender. It was movable (corpus luteum cyst?).

Laryngological examination: The larynx very large; the epiglottis especially. The vocal chords very long, quite livid, with smooth margins. Motility good. The voice is at any rate not too deep and has a very good tone.

X-ray of skull: The thickness and the sclerosis of the frontal bone unaltered.

This time the treatment consisted of encouraging the patient's psyche and zest for work.

Fourteen months after the operation the patient reported by letter that her head hair was not yet natural; she always wore a kerchief over her head. Psychically she was not well, being worried and depressed, but felt considerably relieved since the operation.

DISCUSSION

Only two features of her virilism remained seven to fourteen months after the operation: insufficient growth of hair on the head and a slightly enlarged clitoris. Since the operation the patient has become climacteric and aged. This is undoubtedly due to the cessation of hormone action. It is not to be expected that her menstruation will return at the age of fifty. The vaginal hemorrhage (and enlargement of the left ovary) would have their most natural explanation in the stilbestrol therapy.

Was this patient's virilism pure, or did she present metabolic changes of the Cushing type? There were changes in the carbohydrate metabolism, standard metabolism and in the number of red corpuscles in the blood.

At the first examination there was a slight but definite change in the glucose tolerance curve in the diabetic direction, and the fasting blood sugar value on the whole was rather high. Seven months after the removal of the tumor this change still persisted. Nevertheless, after the operation there were two changes towards the normal: the maximum on the curve was reached fifteen minutes earlier, and the glucosuria was not so protracted. These changes were so slight that one would scarcely assert that they were due to the removal of the tumor.

Prior to the operation the standard metabolism was +33, +14, +17 and +12 per cent of the normal. Seven months after it, the values were −10 and +2 per cent. From being slightly high the standard metabolism had become normal. As the metabolism can

remain high for four to eight weeks after discontinuing the thyreoidin tablets, and as the metabolism perhaps was already falling before the operation, we cannot definitely credit the lower metabolism to the removal of the tumor. It may have been due to the fact that the effect of the earlier, protracted ingestion of thyreoidin was only gradually ebbing out.

Before the operation the number of red blood corpuscles per c.mm. of blood was 5.23 millions, and seven months after the operation 4.50 millions: a doubtful pathological increase before the operation; a doubtful decrease after it. It is particularly true here that more tests before and after the operation would have made the estimate safer.

The conclusion must be that there were slight metabolic changes, but there is no proof that they were due to the tumor, although we cannot exclude that possibility.

COMPARISON OF OWN CASE WITH LITERATURE RECORDS

Kepler, et al. (11) reckon 14 cases of "adrenal-like" ovarian tumors described in all. One was also described by I. Mayer (16). Since then there have been instances published by Greene & Lapp (8) (No. 16), R. Luft (13) (No. 17), Burket & Abell (2) (No. 18). The present case is No. 19. More may have been published; literature is not fully obtainable.

From the descriptions and photographs there seems to be complete agreement in the histological picture in Sellheim's (25), Luft's (13), and the present case, and possibly Bingel's (1) case. These four cases have it in common not to have much lipoid and, therefore, in a morphological sense, they take up an exceptional position among the "adrenal-like" ovarian tumors. The color of the tissue is grey to brownish red. Clinically there was also close agreement, with pronounced virilism, perhaps due to the patients' being about the same age, 49, 44 and 30 years (Bingel: 47 years), and the duration of the affection, about five or six years (Bingel: 12 years). With reference to the Cushing syndrome, the only cases that are fairly well examined are Luft's and the author's. In Luft's case there were no metabolic changes; in the present case there were changes, as already stated, but only few and slight; in Sellheim's there was a little adiposity; in Bingel's a distinct Cushing syndrome. Signs of Cushing's syndrome are not always found in patients with "adrenal-like" ovarian tumors.

After comparing several of the published descriptions of cases of arrhenoblastoma and "adrenal-like" ovarian tumor, we must agree with Kepler, et al., (11) that metabolic changes of the Cushing type have been observed only among the "adrenal-like" cases. On the other hand it is typical that practically no patient with arrhenoblastoma has been examined as regards these symptoms. Consequently we may possibly assume that there was no pronounced Cushing syndrome among the arrhenoblastoma patients, though we cannot deny the possibility that there were isolated symptoms of it. All in all there seems to be a difference in the clinical indications of the two forms of tumor, as Kepler, et al., point out. The question is whether this difference is so absolute as these workers believe.

We know nothing as yet as to where the limits must be drawn for the hormonal effects within the androblastoma series. It is more probable that the hormonal effect is combined with the differentiation of (some) cells in a certain direction than with the circumstance that the tumor is diffuse without tubular regions. Mention has been made of the possibility of differentiation in various directions within the diffuse tumors.

Whatever may be the true fact, it will mean clinical support for Teilum's histologically-based theory that "adrenal-like" ovarian tumors belong to the arrhenoblastomata and are not adrenal cortex tumors, if we can find arrhenoblastomata of common type containing tubular regions, with clinical signs of the Cushing syndrome.

I have been unable to find pure cases of this kind described. The nearest are Canelo & Lisser's case (5), and Kraus's (12), to which I would refer interested readers. As the tumors were only discovered postmortem in both cases, we lack the definitive proof of the causal relation between tumor and signs, i.e., the subsidence of signs after the removal of the tumor.

TABLE 2. ANDROGEN AND 17-KETOSTEROID EXCRETION IN 24-HOUR URINE

Tumor	Lipoids in tumor	Author		Pt.'s age	Androgen		17-Ketosteroid	
					Before operation	After operation	Before operation	After operation
Diffuse:	0	J. Pedersen	1946	50	43 i.u.	5 i.u.	14.5 mg.	3.5 mg.
"adrenal-	0	R. Luft (13)	1944	44			23.8 mg.	
like"	+	Kepler et al. (11)	1944	16			54.6 mg.	2.6 mg.
cells	+	Rottino et al. (22)	1939	23	16 i.u.			
Typical		Mathiesen (14)	1944	24			4 mg.	2.5 mg.
arrheno-		Wijsenbeek et al.	(32)					
blastoma			1940	28	60 i.u.			
(tubules)		Kanter et al. (10)	1940	33	51 i.u.	10 i.u.		
		Westman (31)	1939	16	12 i.u.	2 i.u.		

Hormone Excretion of Virilizing Ovarian Tumors

It is most natural to regard the virilism connected with these tumors as being produced by hormones secreted by their growth. It is surprising to see how few are the tests made for the excretion of hormonal substances. Table 2 shows the investigations made during the past few years. In judging this table it should be borne in mind that the technic in each case was different. Besides, some of the values are averages, others merely single determinations. Finally, the patients are in widely different age groups.

This is a drawback to comparison in the androgen series too, where the results are given in international units.

In those cases where an analysis was made the chorionic gonadotropin excretion was normal, as was that of estrogen. Saphir & Parker (23), however, found an increased quantity of estrogen, about 5000 m.u./1 urine, which subsided after the tumor was removed. In Maxwell's case (15), a 67 year old woman, there was up to 40 m.u. estrogen in the 24 hour urine. The hormone content in tumors has several times been determined (e.g. Wijsenbeek & Plate (32), Kanter & Klawans (10)) and has always been very low.

The few hormone investigations available show that the excretion of steroids in the urine is unexpectedly low considering the marked virilism. The values vary from the normal to the low pathological level. This was already observed by v. Szathmáry (27), the first to examine an arrhenoblastoma for hormones. There may be several reasons for the low excretion, for example, steroids not demonstrable by the methods applied. It is theoretically possible that the relatively low values are fortuitous (the few investigations). The high excretion of estrogen in a few cases makes it presumable that the excretion of steroids is just as variable as with virilizing adrenal cortex tumors.

For the present, these hormone analyses are of only limited value to diagnosis. Definitely increased values argue the presence of a virilizing tumor; normal values do not argue against it; the analysis provides no indication as to the origin (ovary, adrenal cortex, aberrant adrenal cortex). No matter what the value of the excretion of androgen and ketosteroid may be prior to the operation, it seems to fall afterwards, so that it may be of assistance to the diagnosis of a recurrence to follow the excretion of hormones after the operation. In the course of time no small number of these tumors have proved to be malignant, often with a late recurrence (Rottino & McGrath (22), Burket & Abell (2), I. Mayer (16), Kepler, et al., (11)).

Investigations so far are too few to demonstrate any difference in the hormone excretion in cases of diffuse arrhenoblastomata ("adrenal-like" tumors) and arrhenoblastomata of the usual mixed type, and any correlation between hormone excretion and clinical symptoms. As with adrenal cortex tumors, we lack clinically employable methods for the specific demonstration of the various steroids. Until such methods become available, it is advisable to determine both the androgens and the 17-ketosteroids, as there need not necessarily be any parallel between them.

Two curious features are common to gonadal tumors of the androblastoma series and adrenal cortex tumors: they are more frequent in females than in males, and, when they act upon the sexual character, they have a feminizing influence on males and a virilizing effect on females. Why?

Changes in the Endocrine Organs

One of the observations made on our patient was hyperostosis cranialis interna. Four other authors report on changes in the craniogram which may be interpreted as hyperostoses (Novak & Wallis (18), Rottino & McGrath (22), I. Mayer (16) and R. Luft (13)). All these patients had tumors of the "adrenal-like" type. Thus five hyperostoses have been found among about twenty (not all of which were examined for it). If this frequency is correct, it is considerably higher than in the normal population (Pedersen (20)). It seems convincing that in two of the five cases the hyperostosis occurred at the ages of 23 and 32, an age class in which hyperostosis is rare. In three of the cases, the hyperostosis was of the nodular frontalis interna type; in the other two (Novak & Wallis (18) and the author's case) it was hyperostosis intertabularis (20). We do not know the etiology or pathogenesis of hyperostosis, but it is usually regarded as a somewhat diffuse endocrine stigma, indicating changes in the pituitary.

We are unable to decide whether it is of clinical importance that hyperostosis was found only in patients with "adrenal-like" ovarian tumors; only very few of those with arrhenoblastoma of the usual type were x-rayed.

It is also possible to show changes in other endocrine organs. Struma is not uncommon (Geisler (7), Strassmann (26), Wijsenbeek & Plate (32), Bingel (1), Novak & Wallis (18) etc.), and it occurs in both forms of virilizing ovarian tumor.

Fibroma uteri is also frequent in cases of "adrenal-like" ovarian tumors; it occurs in Bingel's (1), Sellheim's (25), Maxwell's (15), Rottino & McGrath's (22) (case 2), Burket & Abell's (2), the author's, and possibly in R. Luft's (13) cases, i.e., in six or seven out of about twenty. A similar frequency is reported for arrhenoblastomata of the usual type (Büttner (3)).

Very few postmortem examinations have been made of patients with virilizing ovarian tumors, and hitherto they have not presented any particularly characteristic changes in the other endocrine organs. In all probability, the explanation of the Cushing syndrome is the secondary action of the tumor product upon other endocrine organs, especially the pituitary.

SUMMARY

The author reports on a case of virilizing, diffuse, non-lipoid containing, "adrenal-like" ovarian tumor in a 50-year-old woman. The tumor could not be palpated by gynecological examination. This is the nineteenth case of virilizing "adrenal-like" ovarian tumor recorded in the literature.

Teilum has demonstrated that histologically there are transitional forms between arrhenoblastomata of the usual type with tubular areas and the "adrenal-like" ovarian tumors, for which reason the latter should be regarded as a variant of an arrhenoblastoma diffusum and not as an adrenal

cortex tumor. Kepler, et al., consider that patients with "adrenal-like" ovarian tumors differ from patients with the usual arrhenoblastoma by having more or fewer symptoms of the Cushing syndrome. The author endeavors to find transitions in the clinical signs of patients with arrhenoblastoma of the usual type and patients with "adrenal-like" tumors, in parallel with the transitions which Teilum has shown histologically. No convincing cases were found, presumably because very few patients with arrhenoblastoma of the usual type have been examined clinically for the Cushing syndrome.

Prior to the operation the present patient excreted an average of 45 i.u. androgen and 14.5 mg. 17-ketosteroid in the 24-hour urine; after the operation 5 i.u. and 3.5 mg. ketosteroid. A survey is given of the hormonal determinations recorded in the literature. The diagnostic value of the hormone excretion in the urine is small, but it may be of importance to the diagnosis of a recurring tumor.

Hyperostosis cranialis interna is a frequent feature in cases of diffuse arrhenoblastoma, whereas the frequency cannot be investigated for those of arrhenoblastoma of the usual type, as x-rays exist in a few cases only. Fibroma uteri and struma are frequent in both forms of virilizing ovarian tumor.

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HYPOGONADOTROPIC EUNUCHOIDISM: REPORT OF CASE WITH FAILURE TO RESPOND TO CHORIONIC GONADOTROPIC HORMONE DUE TO ANTIHORMONES

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THE therapeutic efficacy of most of the available gonadotropic hormone preparations is limited by the rapid development of neutralizing substances generally termed antihormones.

Pregnant mare's serum is particularly prone to call forth the development of these antihormones, thereby restricting the possible usefulness of this therapeutic agent to brief periods. On the other hand, there are available preparations of the chorionic gonadotropic hormone from human pregnancy urine which have appeared to be singularly free of this complicating reaction in man. However, it has been possible to produce neutralizing substances against this human chorionic gonadotropic hormone in various species of animals. This subject has been reviewed by Leathem (5) and Thompson (8).

Despite the lack of demonstrable titers of antihormone against chorionic gonadotropic hormone in man, speculation about its existence has been extensive. A pregnancy test based on the concept that in women antihormones would regularly develop against their own chorionic gonadotropic hormone was proposed (2) but has proved unreliable (3). Furthermore, Leathem and Rakoff (6) failed to find any evidence of such antihormones in a group of postpartum women carefully studied. Fraser and coworkers (1) in Albright's group have suggested that antibodies developed to chorionic gonadotropin from human pregnancy urine, in two patients with hypogonadotropic eunuchoidism (Negative FSH) who had previously been treated with this substance. This suggestion was based upon the observation that these patients failed to respond to administration of this material with an increased output of urinary 17-ketosteroids. No studies for such antihormones were reported.

The case to be described presented a similar problem. Antihormone studies were therefore undertaken.

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CASE REPORT

H.W., a 24 year old white welder, was referred to the Ochsner Clinic because of failure to develop sexually despite gonadotropic hormone therapy.

He had received elsewhere 5 courses of 30 injections each of 1 cc. of Polyansyn[1] with one month rest periods between each course. He had also been given $\frac{3}{4}$ gr. thyroid U.S.P. daily during this period of treatment.

When first seen by us he presented the classical eunuchoid appearance. His span exceeded his height by 2 inches, there was no beard, his voice was high pitched, and axillary and pubic hair were exceedingly sparse. The phallus was extremely small. The testes were in the scrotum and measured about 0.5 cm. ×1 cm. The prostate was very small and no secretion could be obtained on massage.

Roentgenograms of the wrist showed considerable delay in skeletal maturation. On repeated examination the urinary 17-ketosteroids varied between 6.5 and 10.6 mg./24 hrs.

Three attempts to demonstrate gonadotropic hormone in the urine were negative for 4 m.u./24 hrs. by the method of Klinefelter, Albright and Griswold (4). All our normal males have had at least 6.6 m.u./24 hrs. by this method.

Testicular biopsy showed a completely immature testis with no spermatic elements in the tubules and interstitial cells which had the appearance of fibroblasts.

Therapy was initiated with daily subcutaneous injections of 500 I.U. of chorionic gonadotropic hormone from human pregnancy urine.[2] This was continued for six weeks. There was no response clinically. The urinary 17-ketosteroids remained at the pretreatment levels. The range during treatment was 7.8 to 9.4 mg./24 hrs. Therapy was then changed to intramuscular injections of 25 mg. of testosterone[3] in oil daily. There was an immediate feeling of well being, gain in weight and growth of the phallus and prostate. There was a rise in the urinary excretion of 17-ketosteroids—range from 12.0 to 19.0 mg./24 hrs. There gradually followed deepening of the voice and a change to a more aggressive personality.

Studies for antihormones on the patient's serum were carried out on three occasions with the results shown in Table 1. Leathem's technic (6) was employed. Female CFW mice, 23 to 25 days old, were used. Six equal injections were given on three successive days. The gonadotropin[2] and sera were injected separately. Autopsies were performed twenty-four hours after the last injection. As can be seen, there was definitely less response to the chorionic gonadotropin in the animals receiving the patient's serum. Dr. James H. Leathem was kind enough to test the patient's serum for us. He was able to confirm our finding of measurable titers of neutralizing substances against the chorionic gonadotropic hormone from pregnancy urine.

The only other report of antibodies to chorionic gonadotropin from human pregnancy urine of which we are aware is the recent report of Leathem and Rakoff (7). One patient received equine gonadotropin and synapoidin (a combination of sheep anterior pituitary extract and human chorionic gonadotropin) at different times "and the serum when tested was capable

[1] Polyansyn—Armour's polyvalent pituitary extract.

[2] Choriogonin—supplied through the courtesy of Dr. C. A. Miller of Lakeside Laboratories, Milwaukee, Wisconsin.

[3] Testosterone—supplied through the courtesy of Dr. E. G. Upjohn of the Upjohn Company, Kalamazoo, Michigan.

of inhibiting equine gonadotropin and chorionic gonadotropin but did not influence synapoidin." In some of the patients who received synapoidin alone antihormones did not develop. However, in patients in whom antihormones against synapoidin developed it was noted that the antiserum would not antagonize the action of human pituitary but did inhibit equine gonadotropin and chorionic gonadotropin.

TABLE 1. ASSAYS OF PATIENT'S SERUM FOR CHORIONIC GONADOTROPIN NEUTRALIZING ABILITY

Date	Chorionic Gonadotropin International Units	Serum cc.	Number of Mice	Uterine Weight Aver. mg.	Ovarian Weight Aver. mg.
2- 4-16	6	0	5	80.1	4.0
	6	1.25	4	17.2	1.6
3-13-46	20	0	3	60.4	6.1
	20	0.9	3	42.6	3.4
3- 6-46	20	0	4	52.3	5.7
	20	0.9	4	40.5	3.6
Untreated Controls	0	0	16	14.5	2.1

SUMMARY

A case is described of a young man with hypogonadotropic eunuchoidism who failed to respond to adequate therapy with the chorionic gonadotropic hormone from human pregnancy urine. This was demonstrated to be due to the presence of antihormones against this gonadotropic hormone.

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ADDISON'S DISEASE FOLLOWED FOR NINE YEARS: CASE REPORT WITH AUTOPSY

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BECAUSE of the newer methods of treatment, the life of patients with Addison's disease has been extended for several years. The influence this new form of therapy has on longevity, and its effects on body tissues, warrants reporting a case followed for nine years. Furthermore, the fact that this patient's pigmentation underwent marked changes, either spontaneously or because of the therapy, is an additional reason for this report.

This case was previously reported in the October 1940 issue of *Endocrinology* (2), at which time it was summarized as follows:

"A case is reported in a 31 year old male patient who presented all the clinical criteria for the diagnosis of Addison's disease. The patient had been on adrenal cortex extract and sodium chloride, discontinuance of which resulted in an Addisonian crisis. Desoxycorticosterone acetate was given six times a week in 5 mg. doses for two months. Subsequent to this, 600 mg. of desoxycorticosterone acetate, in the form of four pellets of 150 mg. each, were implanted in the abdominal muscles. Marked general improvement, gain in weight, and a substantial increase in blood pressure resulted from this form of therapy. The improvement has been maintained over a period of five months. There has been no noticeable change in the pigmentation of the skin."

An appended footnote to the article stated: "On July 8, (1940), ten months after implantation, he is doing very well without further medication."

At this point it is well to correct an error in the original report. It was stated that there was at that time, July 8, 1940, no noticeable change in the pigmentation of the skin. The photographer made a kodachrome film of the patient on January 8, 1937, six days after admission to the hospital, as he was recovering from the crisis. Also, a regular black and white print was made, reproduction of which is shown in Figure 1. A comparison of the frontal view of Figure 1, made January 8, 1937, with Figure 2, made July 12, 1937 (in kodachrome and published as such in color in *Endocrinology* (2)), shows a marked change in pigment anteriorly.

This change in pigment, marked by its loss in the neck, anterior part

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of the chest, and arms, is readily apparent to anyone. No change is seen in the pigment of the back, Figure 3. My only explanation for such a gross oversight is that the original kodachrome, taken on January 8, 1937, was not nearly as clear in color contrast as those taken on July 12, the latter being the ones used in the original article. Only recently, following the patient's death, when the black and white prints of January 8 and July

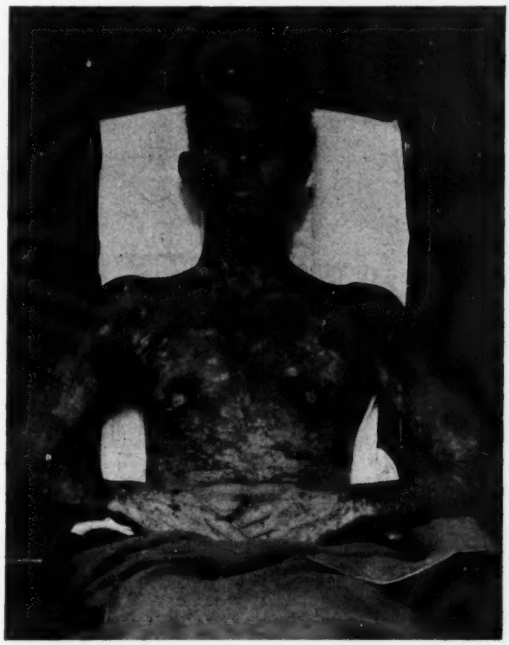

FIG. 1. Photograph made January 8, 1937 showing intense pigmentation with vitiligo or leukoderma of hands.

12 were made, did the contrasting pigmentary changes which came to pass during that time register in my mind. It must be said, however, that both the patient and myself were aware of changes which developed later. Be that as it may, this is no excuse for the clinical oversight.

With this note of apology, in order to date the case, it should be stated that he: "Was first seen on January 2, 1937, with the complaint of abnormal pigmentation of the skin, urticaria, nausea and vomiting, and loss of weight. He always had been dark, he said, but had become much darker during the last eight months. His mother stated that his color did not compare with that previous to his illness. Associated with this, he had

marked weakness, nausea, and vomiting. The nausea and vomiting had been present for one month, but the weakness had been progressing for eight months"; (since approximately April, 1936).

It is of some importance that a maternal aunt died of Addison's disease, the diagnosis having been verified at autopsy. No tuberculosis was found. After the death of the patient, it was learned that the daughter of a brother (patient's niece) was showing pigmentary changes and was to be examined.

The salient features of this patient's subsequent course are as follows:

Photographs made January 23, 1940, that is, three years after he was first seen, showed no change in pigmentation from those of July 12, 1937 (Figures 4 and 5).

One year after the implantation of the pellets, September 7, 1940, he felt well and his blood pressure was systolic 94, diastolic 60. The blood chlorides were 465 mg. He was taking 2 Gm. of sodium chloride daily, but no other medication. His weight was 62.1 kg. (137 pounds). During the original crisis, he weighed 53.1 kg. (117 pounds). When seen February 10, 1941, it was noted that he had lost weight, 2.3 kg. (5 pounds), and his blood pressure was systolic 70, diastolic 62.

He felt nauseated and weak. The blood chlorides were 350 mg., with the recurrence of symptoms seventeen months after the implantation of 600 mg. of desoxycorticosterone acetate. It was felt that this procedure should be repeated, and on February 12 three pellets of 150 mg. each (450 mg.), were implanted in thel eft scapular area. Two Gm. of sodium chloride were taken daily.

On March 28 his blood pressure had reached 122/70. There was some edema of the face and ankles, and the salt was discontinued.

On April 7 he said that he felt fine and the edema had subsided.

The pigmentation of the interscapular areas had begun to fade. Colored movies had been taken soon after recovery from the original crisis in 1937, and now, in April 1941, additional ones were made and these showed loss of pigmentation on the anterior chest, arms, and back. The amount of pigmentary loss was marked.

On September 9 it was noted that there was still more fading of pigment on the back. His blood pressure, however, began dropping, being 100/64. On October 2, about eight months after the last implantation, the mother reported by telephone that he was vomiting, was weak, and was not doing

FIG. 2. Anterior view made July 12, 1937 showing loss of pigment from chest, neck and arms. Urticaria is also present.

FIG. 3. Posterior view made July 12, 1937.

FIG. 4

FIG. 5

FIG. 4. Anterior view January 23, 1940 showing no change in pigment from that of July 12, 1937 (Figure 2).
FIG. 5. Posterior view made January 23, 1940 showing no change in pigment from that of July 12, 1937 (Figure 3).

well. He was seen the next day, at which time he complained of severe headache, marked asthenia, and vomiting. His blood pressure was 86/66. Five Gm. of salt, and 10 mg. of desoxycorticosterone acetate, were given hypodermically. He was sent into the hospital, and with daily injections of aqueous adrenal cortex extract, desoxycorticosterone acetate, intravenous glucose in saline, sodium chloride, vitamin C, and a low potassium and high sodium diet, he recovered rapidly.

An important finding at this time was a severe anemia. The hemoglobin was 58 per cent, 2,860,000 red cells, and only 2,500 leukocytes. The differential count was 60 per cent polymorphonuclears, and 40 per cent lymphocytes. The chlorides were 429 mg. on the second day after admission, following therapy. He was placed on 5 mg. of desoxycorticosterone acetate daily, 5 Gm. of sodium chloride and .2 (3 grains) of ferrous sulfate four times a day. The latter drug produced watery stools and had to be discontinued, but was resumed one week later with three doses per day. His blood count improved, and on October 24 hemoglobin was 73 per cent, 3,930,000 red cells and 5,500 leukocytes, with a normal differential count.

He was seen at monthly intervals with no marked change in his condition. The blood pressure fiucutated between 100/64 and 124/80.

He did well until June 13, 1942, when he again complained of weakness, vomiting and severe headache. He said the salt caused the headache, and had discontinued it May 1. His blood pressure was 100/62. Another implantation of 525 mg. of desoxycorticosterone acetate pellets was made in the left scapular area on June 15. The total amount of pellets implanted was 1,575 mg.

By June 26 he was once more on the road to recovery with a blood pressure of 124/80. He was taking 200 mg. of vitamin C and .6 (9 grains) of ferrous sulfate per day. His appetite improved.

On November 28, he had a chill, fever and weakness. Five mg. of desoxycorticosterone acetate and 2 Gm. of sodium chloride were given daily.

He progressed well on this medication until June 10, 1943, when on his visit to the office, he stated that he was tired of giving himself hypodermics, and it was decided to use sublingual desoxycorticosterone acetate in propylene glycol. A dosage equal to 3 mg. was given sublingually. In addition, 1 Gm. of sodium chloride was also given.

He developed an upper respiratory infection on October 4, and his blood pressure dropped to 80/48. A double dose of the sublingual medication was given and the sodium chloride increased. He occasionally felt weak and shaky and it was thought that hypoglycemia was present, but no blood sugar studies were made.

FIG. 3. Posterior view made January 28, 1940 showing no change in pigment from that of July 12, 1937 (Figure 3).

His pigment continued to fade and on April 10, 1944, photographs were taken (Figures 6 and 7).

The leukodermic areas around the eyes were especially noticeable, and increased his bizarre appearance. Very little pigment remained anteriorly except on the face and neck. The pigment on the back and arms had also largely disappeared except on the neck and a few areas on the shoulders and arms.

On December 17, 1944 he said that he was weak and his blood pressure was 100/82. He was given, in addition to the sublingual medication, 2 cc. of adrenal cortex extract. He complained that his mouth watered a great deal, and on February 9, 1945 the submaxillary glands were swollen to approximately twice their normal size and salivation was troublesome. The sublingual cortate was stopped, and the adrenal cortex was increased to 4 cc. per day.

By March 2, the salivary-gland swelling had subsided and the salivation disappeared.

On July 16 he returned to a daily dose of 5 mg. desoxycorticosterone acetate. He continued this therapy, and on May 14, 1946 he did not look as well as previously. His blood pressure was 90/64.

In the early part of July he went to a summer cottage on a lake near Detroit, and his family noted that he was acting peculiarly. He made the the statement that he was tired of his illness and tired of giving himself "shots." He did not take his medication for two weeks. He became reticent, irritable, and wanted to be alone. On July 23 he became delirious, had a temperature of 101 to 103, and he was sent to Harper Hospital on July 27 in a comatose condition. His blood pressure was 96/70. The hemoglobin was 11.5 Gm., 75 per cent, red cell count 3,550,000, leucocytes 9,900, with a normal differential count. Fasting sugar was 32 mg. and nitrogen 48 mg.

Intravenous glucose, 10 cc. adrenal cortex extract every 4 hrs., and 25 mg. desoxycorticosterone acetate, did not bring him out of the crisis. He remained comatose and his temperature rose rapidly to 106. He became pulseless. His blood pressure could not be obtained. He died July 28, at 3:00 p.m., the day after admission.

The autopsy was performed one hour later.*

External examination: The body was that of a 41 year old white man, moderately well-developed and nourished, about five feet, seven inches tall and weighing about 130 pounds. The hair was thick and black. The face and neck were deeply pigmented with dark tan coloration, except for

* My thanks are due Dr. Plinn F. Morse and Dr. Herbert W. Devine for the reports on the autopsy.

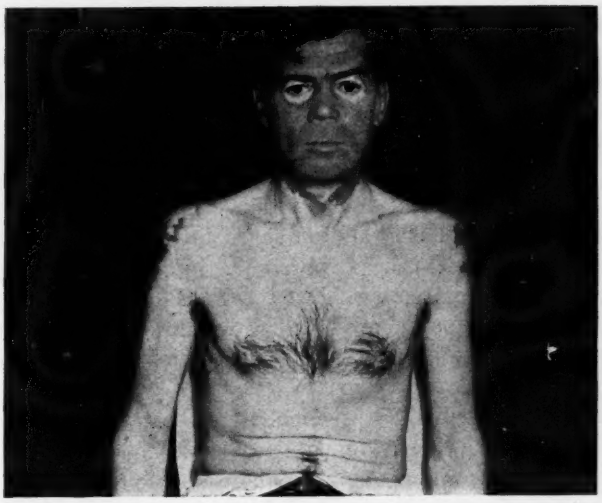

FIG. 6

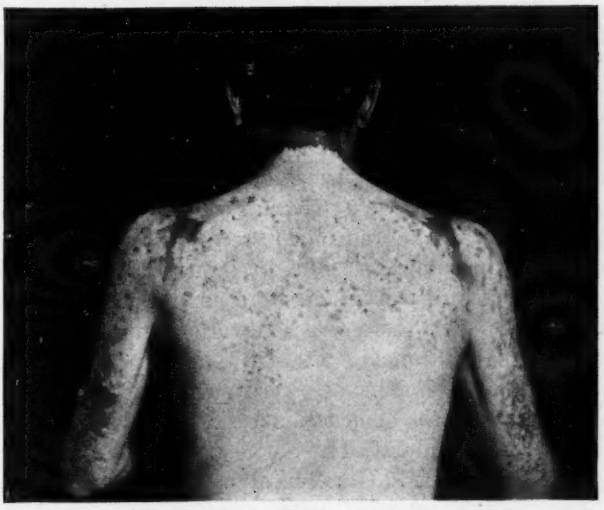

FIG. 7

FIG. 6. Anterior view made April 10, 1944 showing further loss of pigment from arms. Vitiligo around eyes is well shown.

FIG. 7. Posterior view made April 10, 1944 showing marked loss of pigment from back, arms and neck.

small areas about both eyes and the sides of the nose. The eyes were equally dilated. The nose and mouth were normal. The teeth were in fair repair. No pigmentation was seen in the mucous membranes. The ears were thickened and quite rigid in character. The pinnae felt as though they were partially ossified. The thyroid was not palpable in the neck. The neck appeared normal except for the previously described pigmentation. The chest was moderately well developed and resonant bilaterally. There was a one-inch-long scar in the left abdominal region, slightly above the umbilicus and lateral to it. This was the site of implantation of desoxycorticosterone. The upper extremity showed freckles of deep tan pigmentation, while the rest of the body showed marked pallor. There was no evidence of old or recent injury to the extremity. Rigor mortis was slight and livor mortis only moderate.

Main incision: The body was opened by the usual Y incision. There was about 1 cm. of white panniculus. The tissues appeared to be dry. The musculature was well developed. There was no fluid in the abdominal cavity. The peritoneum was normal and the organs were in their normal anatomic relations.

The sternum was removed by cutting the costal cartilages. The pleural cavities were dry. No adhesions between parietal and costal pleura were present. The pericardium was opened *in situ*, revealing a small heart. There was about 30 cc. of clear yellow fluid in the sac, which itself was normal and shiny.

The pulmonary artery was opened *in situ* revealing no clot or thrombus. The great vessels of the neck were ligated and the thoracic structure removed *en masse*. The right lung weighed 430 Gm.; the left 400 Gm. They were blue-gray in color and were pneumatized. Both floated well in water. The bases of both lungs showed moderate hypostatic congestion, and on section were wet. On compression in the larger bronchioles, thick yellow secretion could be expressed. There were small calcified glands in both hili and at the left apex there was a fibrotic scar without calcification. The trachea and major bronchi contained a small amount of thick, yellow secretion. The mucous membranes were normal throughout. The aorta showed no atherosclerosis. The coronary arteries were patent at their origin and normal throughout. The aortic valves were normal; the heart was quite small, weighing 210 Gm. The myocardium appeared normal. The ventricles and auricles showed mild postmortem clot, but otherwise showed no abnormality. The tricuspid, mitral, and pulmonary valves were normal. The esophagus showed no abnormality. The organs of the abdomen were in their normal anatomic relation. The liver was small and light red in color; it weighed 1,350 Gm. externally, and on section it showed

normal architecture. The diaphragm was intact. The gall bladder was long and pendulous. It held about 50 cc. of brown bile and contained numerous small pigmented (sand) granules. The mucosa appeared normal. The spleen weighed 240 Gm. and was slate blue in color. On section it showed normal architecture. The pancreas was small and weighed only 50 Gm. On section it showed normal pink-gray lobulated tissue, and the ducts and vessels were of normal consistency. The adrenals were absent, even after extensive dissection above the kidneys down as far as the pelvis. No demonstrable adrenal tissue could be found, even after the perinephritic fat was minutely sectioned grossly. Accessory adrenal tissue was hunted for down the abdominal aorta and along the spermatic vessels, but none was found. The perinephritic tissue was placed in formalin for further investigation. The kidneys were small, the right weighing 70 Gm., the left 80 Gm. They were slightly lobulated, and the left kidney had an old, well-healed infarct in the inferior pole. The capsule was stripped with moderate difficulty. The cortex was thin and petechial; hemorrhages were demonstrated in the left pelvis. The ureters were normal. The bladder was opened *in situ* and showed normal structure. The prostate was small and normal. The stomach was opened, showing postmortem autolysis but no other change. The duodenum, small and large bowel, were normal throughout. The thyroid was dissected out, and the lobes were small and light pink in color. The testes were removed. They also were small but normal in structure.

Head: The scalp was incised in the usual fashion and the calvarium removed. The dura was normal. The piarachnoid was normal. The brain weighed 1,535 Gm., and showed no abnormality externally. The lateral ventricles were opened and were normal. The cerebral vessels showed minimal or no evidence of sclerosis. The brain was placed in fixing solution for further study.

Gross anatomic diagnosis:

1. Absence of both adrenals grossly.
2. Extensive pigmentary changes of the skin of the face, neck, shoulders and arms.
3. Cholelithiasis.
4. Small kidneys with fetal type lobulation, and an old, healed infarct of the left kidney.

Microscopic diagnosis:

1. The pituitary showed diffuse fibrosis of the whole anterior lobe with curious acinar development resembling thyroid acini. The acinar cells had

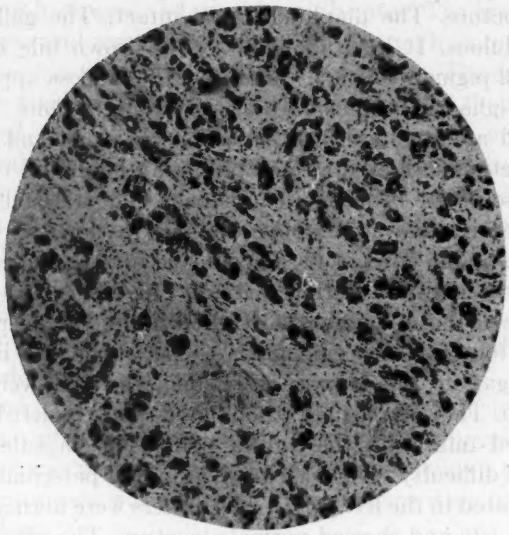

FIG. 8

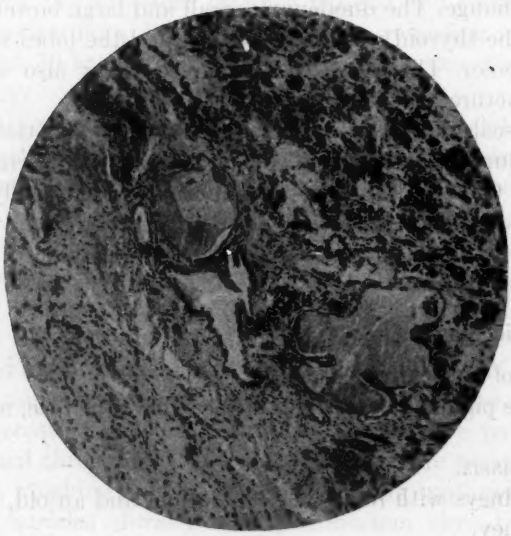

FIG. 9

FIG. 8. Photomicrograph of pituitary (×125) showing diffuse fibrosis with acinar development resembling thyroid acini. Basophilic cells predominate.

FIG. 9. Section of pars intermedia (×125).

a basophilic cast. The pars intermedia showed no notable morphological change. The pars nervosa appeared normal except for thickening of the capsule (Figures 8 and 9).

2. No evidence of adrenal tissue was found.

3. The thyroid showed extreme thyroid exhaustion, with marked lymphoid infiltration as well as degenerating fibrosis (Figure 10).

4. The lungs showed generalized emphysema and septal atrophy. They had the appearance of generalized mesodermal weakness. There was chronic pleuritis with low grade anthracosis, which were not clinically significant (Figure 11).

5. The kidneys showed slight indication of a presenile atrophy; otherwise there were no morphologic changes.

6. The pancreas showed no notable morphologic changes. There was plenty of insular tissue.

7. The liver showed stasis about the central veins, probably due to cardiac stasis. A small amount of infiltration with lymphocytes into Glisson's island was present. Otherwise, the liver appeared normal.

8. The perirenal fat showed atrophied fat tissue with collapsed cells, resembling that seen in aged persons.

9. The gall bladder showed postmortem autolysis.

10. A sympathetic ganglion was found in the perirenal fat which showed no notable morphologic changes.

11. The testes showed no notable morphologic changes except slight thickening of the tunica vaginalis (Figure 12).

12. The heart showed general atrophy but no fibrosis or sclerosis (Figure 13).

13. The brain showed no sclerosis of the vessels in the piarachnoid, although there was a small petechial hemorrhage in this area (Figure 14).

14. The spleen showed moderate splenic fibrosis as well as a senile type of atrophy.

15. The diaphragm showed no notable morphologic change.

16. The aorta showed one moderately large patch of atheromatous sclerosis.

COMMENT

The case presents several interesting features which are subject to various interpretations. The clinical diagnosis of Addison's disease was substantiated by the autopsy, and as noted, not a trace of adrenal tissue was found.

How long would this patient have lived without therapy? We know that it is not unusual for patients to live this long and longer without much therapy, but the total absence of the adrenals, and the fact that negligence

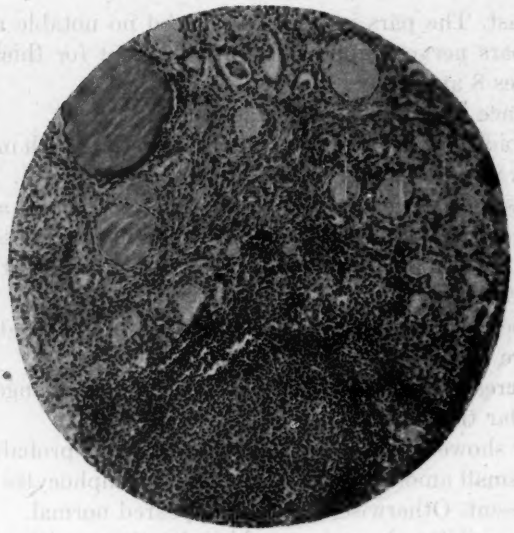

FIG. 10

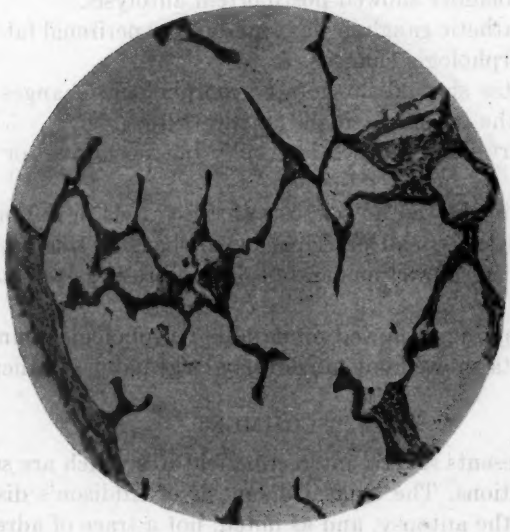

FIG. 11

FIG. 10. Section of thyroid (×125) showing exhaustion, marked lymphoid infiltration and degenerating fibrosis.

FIG. 11. Emphysema of lung (×125) with septal atrophy.

FIG. 12

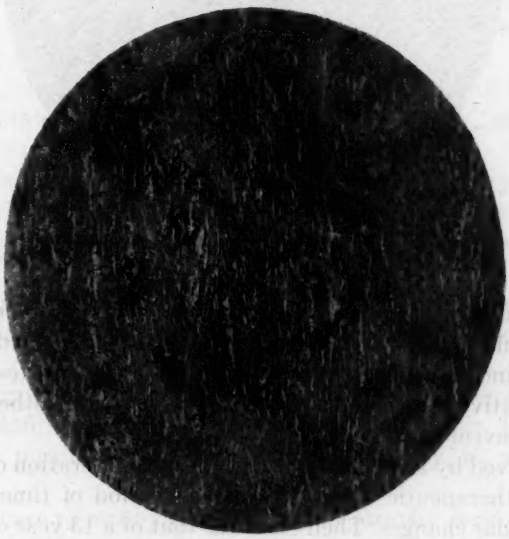

FIG. 13

FIG. 12. Section of testicle (×125) showing normal structure.
FIG. 13. Section of heart muscle (×125) showing atrophy.

of therapy resulted in episodes of Addisonian crises and finally in death in one of these periods, lead to the supposition that the medication kept him alive. Such a severe involvement of the adrenals is, in itself, evidence that the therapy had prolonged the patient's life.

It seems justifiable to suppose that there was an anlage defect of the adrenals since a maternal aunt died of Addison's disease, the diagnosis having been verified by autopsy. Lending further support to this supposition is the fact that a niece of the patient (a brother's daughter) was showing pigmentary changes in the skin. It was this latter circumstance which as-

FIG. 14. Section showing hemorrhage in piarachnoid (×125).

sisted in obtaining the autopsy for we, and the family, were interested in knowing the effect desoxycorticosterone acetate therapy had on the tissues, particularly since a recent report by Forster and associates (1) had found some degenerative lesions in the cerebral arteries while other arteries studied failed to reveal any changes.

It was believed by these authors that the administration of the hormone, in moderate therapeutic doses over a long period of time, produced the cerebral vascular changes. Their case was that of a 13 year old boy, treated for two years. The patient herein reported received the drug for seven years without cerebral vascular changes, unless one includes the small hemorrhage in the piarachnoid as such a vascular change. Sections of the

cerebrum, and other areas of the piarachnoid, did not show vascular changes. Selye, Beland and Sylvester (5) described changes of the cerebral blood vessels in rats treated with large doses of the hormone, and salt.

The change in pigmentation is of great interest. The pigmentation anteriorly on the chest, neck and arms had already begun to fade within six months after beginning therapy with aqueous cortical hormone, salt and vitamin C. Rothman (4) said that control of the patient with cortical hormone and salt therapy is without appreciable effect on the pigmentation.

Attention has already been drawn to my oversight in the loss of pigment within the first six months. That there was a striking loss of pigment cannot be denied, as shown by the photographs, the colored movies, and the observations of my colleagues as well as the patient himself.

It is evident, however, that vitiligo or leukoderma is not an uncommon finding in Addison's disease, and whatever the mechanism is that produces the vitiligo may also be responsible for the loss of pigment.

Certainly no claim can be made that the therapy was responsible. I was of the opinion that the pigmentation of Addison's disease was due to the activity of the pars intermedia of the pituitary. The reason for this was given in an article by Bates and myself (3). The pars intermedia in this case showed no notable morphologic changes, and until such time as physiologic chemists can provide satisfactory clinical tests *in vivo* of this hormone, speculation is useless.

In the above mentioned article (3) we reviewed some of the literature relative to the state of the pituitary in Addison's disease, as well as after extirpation of the adrenals in animals.

Most autopsies of Addison's disease, in which the pituitary was studied, showed degenerative changes (3). There is evidence (3) that the pituitary attempts to compensate for adrenal cortex atrophy, but eventually undergoes atrophy.

The fibrosis of the whole anterior lobe with acinar development, resembling thyroid acini, is of interest. The cells were practically all basophilic.

The changes in the thyroid gland were those of an extreme exhaustion, with lymphoid infiltration as well as degenerating fibrosis. How much of this was due to pituitary stimulation, and to therapy, is, of course, unknown.

The lung changes of emphysema and septal atrophy seem to be part of the general exhaustion and weakness of tissues, and as expressed by the pathologist, Dr. Morse, "mesodermal weakness." No evidence of tuberculosis was found.

It is of interest that there was an abundance of pancreatic insular tissue. The low blood sugar found antemortem of 32 mg. certainly is a severe hypoglycemia, but no convulsions were present.

What of the pituitary-pancreatic-adrenal cortex relationship to the hypoglycemia, and the states of these glands during the life of the patient? Was there sufficient pituitary activity to stimulate the pancreatic insular tissue and thus produce hypoglycemia, or was there insufficient contrainsular hormone in the pituitary?

It is pure speculation, of course, whether the therapy with the 17-ketosteroids had anything to do with the formation of cholelithiasis, or whether the disease itself produced a change in cholesterol metabolism. Only one moderately large-sized patch of atheromatous sclerosis was found in the aorta.

The testes showed no morphologic changes of consequence, although they were grossly small. Potency had been restored following therapy, but in the later years he was reluctant to discuss his potency. He remained a bachelor.

SUMMARY

A case is reported of a 41-year-old male who had been followed for a period of nine years, and who had symptoms of Addison's disease for ten years. He had been treated with aqueous adrenal cortex extract, vitamin C, salt, implantation of 1,575 mg. of desoxycorticosterone acetate pellets, and intramuscular injections of this hormone, as well as by sublingual application. The latter method resulted in swelling of the salivary glands and salivation.

A change in pigmentation, previously unnoticed, occurred within six months after therapy with adrenal cortex extract and salt. Over a period of nine years, there was a marked loss of pigment. No claim is made that therapy was responsible for this loss of pigment.

Several episodes of Addisonian crisis occurred, requiring a stepping-up of treatment dosage. Failure to take treatment for two weeks resulted in an Addisonian crisis from which he did not recover. Exitus was preceded by delirium, fever, and hypoglycemia (blood sugar 32 mg.).

At autopsy, no adrenal tissue was found anywhere, and it was suggested that inasmuch as a maternal aunt had died of Addison's disease (verified at autopsy), and that a niece (a brother's daughter) was developing pigmentation, an adrenal anlage defect was postulated.

In addition to the absent adrenals, the important autopsy findings were:

1. The pituitary showed the following: diffuse fibrosis of anterior lobe with curious acinar development, resembling thyroid acini; acinar cells

showing a basophilic cast; no changes in the pars intermedia; a thickened capsule of the pars nervosa.

2. The thyroid showed extreme exhaustion with degenerating fibrosis.
3. The lungs showed emphysema and a septal atrophy.
4. The pancreas had plenty of insular tissue.
5. Cholelithiasis was present.
6. The testes were small grossly, but normal morphologically.
7. The heart was small and showed general atrophy.

It is regretted that a section of pigmented skin which was removed at autopsy was lost.

ACKNOWLEDGMENT

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CO-EXISTING MYXEDEMA AND HYPERPARATHYROIDISM: CASE REPORT

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THE following case report is presented because in the first place it possesses the unusual feature of thyroid and parathyroid disease occurring in the same patient. In the second place it demonstrates many medical problems which emphasize the importance of considering hyperparathyroidism in syndromes with gastrointestinal symptoms. Finally, it suggests a psychosomatic problem: the relationship of alterations in calcium and phosphorus metabolism to the development of a major psychosis.

CASE REPORT

The patient was a white female who came under observation for the first time in 1936 at the age of 50. Her complaints at that time were shortness of breath and palpitation of ten years' duration. There had been no recent increase in the severity of her symptoms but she was known to have had cardiac enlargement for ten years and had been previously regarded as suffering from arteriosclerotic heart disease.

Her past history was a full one. At the age of seven she had malaria. She was treated for tuberculosis in adolescence and for a possible recurrence at the age of twenty-five because of night sweats and chest pain. A tonsillectomy had been performed in childhood. A hemorrhoidectomy was done in 1913. There had been a salpingo-oophorectomy in 1918. A lipoma of the breast was removed in 1924 and a pelvic repair with amputation of the cervix was performed in 1936. Menopausal symptoms began at the age of 35 but menstruation continued until the age of 40. She had several miscarriages, her first and third pregnancies ending spontaneously at 3½ months.

On questioning, a history was elicited of loss of hair of several years' duration, drowsiness, and occasional swelling of the ankles. She also stated that she was extremely sensitive to cold.

Physical examination in June 1936 showed the hair of the scalp to be sparse but not especially coarse. The axillary hair was also sparse, but there was no loss of the outer third of the eyebrows. The pupils reacted briskly to light and accommodation. The neck was normal. The lungs were clear. The heart sounds were normal, there were no murmurs, and the blood pressure was 128/76. There was a slight scoliosis. The left knee was swollen, and there were bilateral bunions. The ankles were slightly edematous. Roentgenoscopy and a roentgenogram of the chest showed slight generalized cardiac enlargement. The hemoglobin was 75 per cent (Sahli). The electrocardiogram was within normal limits. Urinalysis showed a specific gravity of 1.012, no albumen or reducing substance and a normal sediment.

The diagnosis of myxedema was considered because of the presence of long-standing, non-progressive dyspnea, sensitivity to cold, loss of hair, drowsiness, cardiac enlargement

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and anemia. The basal metabolic rate was determined and was found to be minus 38 per cent. The patient was started on thyroid medication. She proved extremely sensitive to the drug, developing severe headache and palpitation, as is usual in myxedema, so that the initial doses had to be small. The dosage was increased gradually from 15 to 130 milligrams daily, with prompt improvement in her symptoms. In the spring of 1937, her bunions were operated on with a good result. In October 1937, she had an attack of

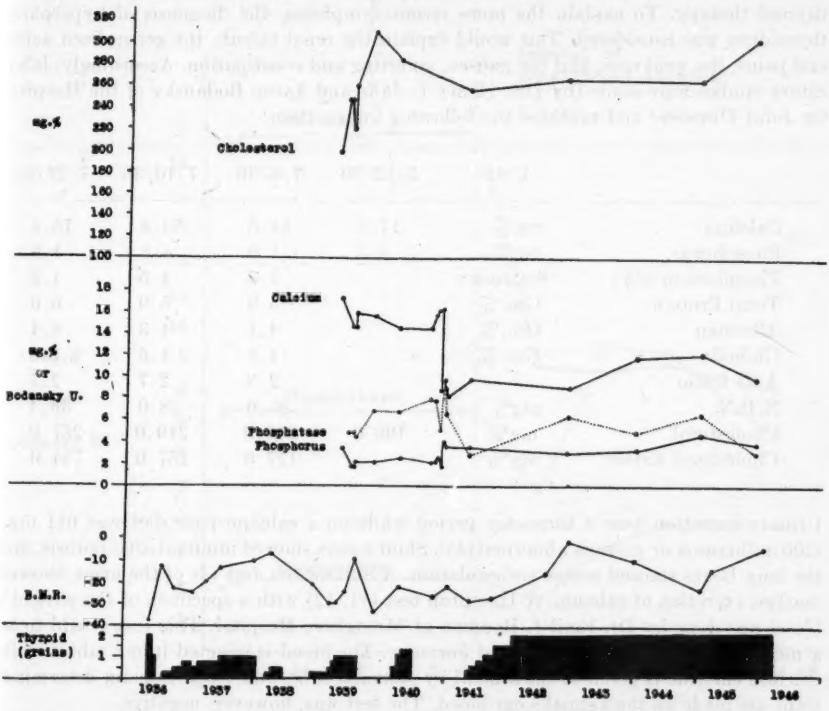

FIG. 1. Composite chart of the pertinent biochemical findings, the basal metabolic rates, and thyroid medication for the entire period of observation.

sciatica, but otherwise felt well. Her hair had stopped coming out, palpitation on effort was less, and her hemoglobin rose to 90 per cent. She was able to withstand cold without discomfort, and was no longer drowsy. Swelling of the ankles disappeared, and a roentgenogram of the chest showed a small but definite decrease in the size of the heart. The dyspnea, however, did not improve.

In December 1937, she developed a rectal fissure which required surgical treatment. In January 1938, she had an attack of "indigestion," with abdominal pain, vomiting, and passage of mucus by rectum. She was seen by a gastroenterologist who made a diagnosis of of mucous colitis. During the summer of 1938, she developed chills and fever, pain in the left loin, and pyuria. An x-ray film showed the left kidney to be the site of many

calculi. Operation was advised but was delayed until January 1939, at which time a left nephrectomy was done for calculous pyonephrosis.

For a short time after the operation she felt better, but then began to complain of increasing constipation, frequent nausea and vomiting, aches and pains in various parts of the body, and weakness. Physical examination revealed no obvious cause for her complaints. There was no evidence of peripheral neuritis or arthritis, no anemia, and her BMR was minus 10 per cent. Her new complaints were clearly not due to insufficient thyroid therapy. To explain the more recent symptoms, the diagnosis of hyperparathyroidism was considered. This would explain the renal calculi, the generalized aches and pains, the weakness, and the nausea, vomiting and constipation. Accordingly, laboratory studies were made (by Drs. Henry L. Jaffe and Aaron Bodansky of the Hospital for Joint Diseases) and revealed the following information:

	Units	5/12/39	7/6/39	7/10/39	7/27/39
Calcium	mg%	17.0	14.5	14.4	15.7
Phosphorus	mg%	3.3	1.9	1.8	1.9
Phosphatase (alk)	Bodansky		4.5	4.5	4.3
Total Protein	Gm.%		5.9	5.9	6.0
Albumen	Gm.%		4.1	4.3	4.4
Globulin	Gm.%		1.8	1.6	1.6
A/G Ratio			2.3	2.7	2.7
N.P.N.	mg%		31.0	28.0	39.4
Cholesterol	mg%	196.0	245.0	219.0	257.0
Cholesterol Esters	mg%		177.0	157.0	184.0

Urinary excretion over a three-day period while on a calcium-poor diet was 611 mg., (200 milligrams or more is abnormal (4)). Skull x-rays showed minimal osteoporosis, and the long bones showed coarse trabeculation. A Sulkowitch test (3) of the urine showed marked excretion of calcium. A Hamilton test (11, 12) with a specimen of the patient's blood was done by Dr. Emil J. Bauman of Montefiore Hospital. This test is said to be a measure of circulating parathyroid hormone. The blood is injected into a rabbit while calcium chloride is given to the animal by stomach tube, and blood calcium determinations are made on the animal's ear blood. The test was, however, negative.

An electrocardiogram taken in 1933 showed a short QT interval. The actual figure was 0.32 second, the R-R interval 0.99. According to Bazett's formula (5), systole = $K\sqrt{\text{cycle}}$, in which K in women is $.40 \pm .04$, the QT interval should have been 0.36 to 0.44 second. Or expressed in the way that Barker, Johnston and Wilson (2) have suggested, K was .32, whereas it should have been $.40 \pm .04$. According to Ashman's formula (1) $QT = K \log [10 (C + k)]$ in which C = cycle length, K = .385 to .390, and k = 0.07, the figure should have been .484 to .49. Other electrocardiograms taken in June 1936 and in April 1940 showed similar shortening of the QT interval so often seen in cases of hypercalcemia (14).

With all these evidences of hyperparathyroidism, an exploratory operation on the neck was performed on November 9, 1939, but no tumor of the parathyroids was found. Mediastinal exploration was advised but the patient refused. In the ensuing year, the persistence of the symptoms and blood chemistry findings indicated that she still had hyperparathyroidism, and on November 19, 1940 the patient was seen by Dr. Fuller

Albright at the Massachusetts General Hospital. The diagnosis was confirmed, and four days later a re-exploration of the neck was carried out by Dr. Oliver Cope but again without revealing any tumor of the parathyroids. A small papillary cystadenoma of the thyroid gland was removed, along with a specimen of the thyroid gland. The thyroid itself was normal on gross and microscopic examination. The adenoma was reported as "long-standing adenomatous goiter with calcification and secondary papillomatous

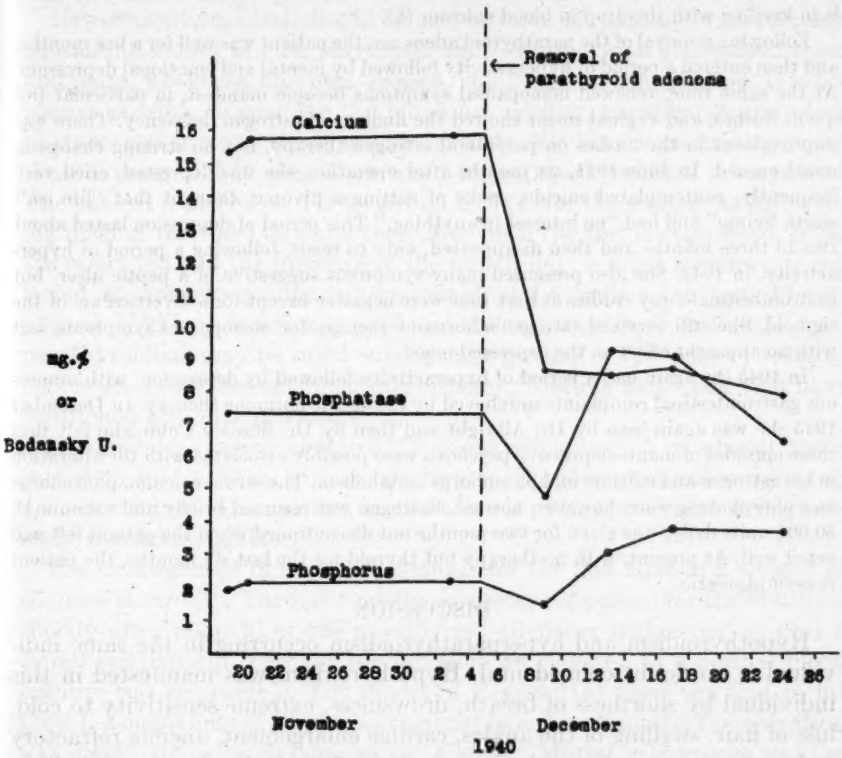

FIG. 2. Studies of blood calcium, phosphorus and alkaline phosphatase in the periods immediately before and after removal of the parathyroid adenoma.

change." On December 5, 1940, a sternum-splitting operation was performed; a parathyroid adenoma was located in the upper portion of the thymus gland and removed. Blood studies during the preoperative and postoperative periods are presented graphically in the accompanying charts. The pathologist's report was of a "tumor $1.8 \times 8 \times 0.5$ cm. weighing 0.4 Gm. Parathyroid adenoma, glandular and cystic type." (8)

The postoperative course was marked by an incomplete atelectasis of the lower lobe of the left lung, and by a mediastinitis. There were symptoms of mild tetany, manifested by numbness and tingling of the fingers and feet, and a positive Chvostek sign, but these

disappeared by the seventh day. The rest of the course was uneventful, and the patient left the hospital on the twenty-third day after the second operation.

Subsequent follow-up revealed that, whereas the symptoms of hyperparathyroidism had disappeared, the picture of hypothyroidism remained unchanged. The patient had remained on a maintenance dose of 130 milligrams of thyroid daily, and the symptoms of myxedema had been controlled satisfactorily. An electrocardiogram on May 24, 1946 showed an increase in the QT constant of Bazett (5) to .41 (normal $.40 \pm .04$), which is in keeping with the drop in blood calcium (8).

Following removal of the parathyroid adenoma, the patient was well for a few months, and then entered a period of hyperactivity followed by mental and emotional depression. At the same time, renewed menopausal symptoms became manifest, in particular frequent flushes, and vaginal smear showed the findings of estrogen deficiency. There was improvement in the flushes on parenteral estrogen therapy, but no striking change in mood ensued. In June 1941, six months after operation, she was depressed, cried very frequently, contemplated suicide, spoke of getting a divorce, thought that "life isn't worth living," and had "no interest in anything." This period of depression lasted about two to three months and then disappeared, only to recur, following a period of hyperactivity, in 1942. She also presented many symptoms suggestive of a peptic ulcer, but gastrointestinal x-ray studies at that time were negative except for a diverticulum of the sigmoid. She still received estrogenic hormone therapy for menopausal symptoms, but with no apparent effect on the depressed mood.

In 1945 she again had a period of hyperactivity followed by depression, with numerous gastrointestinal complaints unrelieved by estrogenic hormone therapy. In December 1945 she was again seen by Dr. Albright and then by Dr. Stanley Cobb who felt that these episodes of manic-depressive psychosis were possibly associated with the alteration in her estrogen and calcium and phosphorus metabolism. The serum calcium, phosphorus and phosphatase were, however, normal. Estrogen was resumed briefly and vitamin D, 50,000 units daily, was given for two months but discontinued when the patient felt and acted well. At present, with no therapy but thyroid for the last six months, the patient is asymptomatic.

DISCUSSION

Hypothyroidism and hyperparathyroidism occurring in the same individual is no doubt coincidental. Hypothyroidism was manifested in this individual by shortness of breath, drowsiness, extreme sensitivity to cold, loss of hair, swelling of the ankles, cardiac enlargement, anemia refractory to iron therapy, an elevated serum cholesterol, and a low basal metabolism, all improving after thyroid medication. Hyperparathyroidism, while probably present even in 1933, judging by the short QT interval in the electrocardiogram, could hardly account for all the symptoms and signs. In fact, measurement of the basal metabolic rate in several cases of hyperparathyroidism was normal (9). Nevertheless it must be noted, in spite of such manifest symptoms of hypothyroidism, that except for the small papillary cystadenoma with calcification, the thyroid gland of this individual was normal both grossly and microscopically. One may merely speculate that the pituitary thyrotropic hormone in this patient was decreased.

The vomiting and constipation were the result of the hypercalcemia which existed prior to the removal of the parathyroid adenoma. That vomiting may be a prominent symptom in hypercalcemia should be emphasized. Recently, Rogers (15) reported two cases of hyperparathyroidism in which peptic ulcer was suspected but not found, and in which hyperparathyroidism was found only at necropsy.

Hyperparathyroidism should be suspected in all patients with calcium-containing kidney stones, polydipsia and polyuria, with generalized aches and pains, weakness, anorexia, nausea, vomiting and constipation. It is not always possible to examine the blood for calcium, phosphorus and phosphatase, and to x-ray the bones of the body. We have been making use of the following simple procedure when hyperparathyroidism is suspected. The patient is placed on a low-calcium diet (4) for three days. At the evening meal of the third day and thereafter until the test is completed, fluids are restricted. On the morning of the fourth day, a urine specimen is collected, its specific gravity is determined, and a Sulkowitch test (3) performed. If kidney function is good and the test reveals no calcium, hyperparathyroidism may be ruled out. Specific gravity of the urine of 1.022 or higher serves to rule out renal insufficiency. Keating and Cook (13) have mentioned a similar procedure.

Sulkowitch's reagent contains 2.5 grams oxalic acid, 5 cc. glacial acetic acid made up to 150 cc. with distilled water. Equal amounts of reagent and urine are mixed. The calcium in the urine is precipitated almost immediately as calcium oxalate.

The development of a cyclic psychosis for the first time in a woman of 55 years is rare (7). The fact that the symptoms appeared for the first time shortly after removal of the parathyroid adenoma makes one suspect a causal relationship. Perhaps the fall from an elevated to a normal blood calcium was responsible. The report of Rogers (15) mentions that in his Case 1, a negro man of 36, the psychiatrist found antemortem (no operation was done) "an agitated depression." Captain Martel, whose case was the first of hyperparathyroidism diagnosed in this country, was known to have changed his behavior after removal of his adenoma. From a calm individual, he became an apprehensive and agitated one. This was attributed to the sudden drop in his blood calcium. Hypoparathyroidism may be associated with major psychosis (6). It is possible that further information about manic-depressive psychosis may be obtained from long-term studies of calcium absorption and excretion. Some variability in the serum calcium levels in patients with psychoneuroses and psychoses has been found (6) but no conclusive evidence has been presented as yet.

SUMMARY

1. A case of hypothyroidism and hyperparathyroidism, occurring simultaneously in the same individual, is presented.

2. The importance of gastrointestinal symptoms, namely, anorexia, nausea, vomiting and constipation, in hyperparathyroidism is emphasized.

3. A simple urine test of calcium excretion with Sulkowitch's reagent, following a 3 day low calcium diet and a half-day of restricted fluids, is suggested as a method of ruling out the presence of hyperparathyroidism. A negative calcium excretion with good kidney function speaks against hyperparathyroidism.

4. A manic-depressive psychosis followed removal of the parathyroid adenoma. This might have been due to the sudden change in calcium and phosphorus metabolism.

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THE HYPEREMIA AZT AND THE EVALUATION OF THE HYPEREMIA RAT UNIT OF CHORIONIC GONADOTROPIN*

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INTRODUCTION

IN an earlier communication (7) we reported the results of 300 rapid pregnancy tests. Infantile female rats, which respond in a few hours to the presence of prolan B (luteinizing hormone—LH) with anterior pituitary reaction (APR) II, served in these tests as the experiment animals. APRII in the rat takes the form of a marked hyperemia, and is essentially different in character from APRII in the mouse and rabbit where prolan B induces follicle hemorrhages (blood points) (3).

The hyperemia AZT has been used routinely in this laboratory in a total of 2500 examinations. The test has proved to be a very reliable one, and in actual practice has completely replaced mouse (original AZT) and rabbit (Friedman's modification of AZT) tests. The error of the twenty-four hour hyperemia test, when the technic is sufficiently known, does not exceed that of the older AZT methods, i.e., 0.5-1 per cent. The two and six hour hyperemia tests, on the other hand, showed in our rats error rates of 31 per cent and 8 per cent respectively.

In developing the hyperemia test it became desirable to determine the minimum dose of prolan B (LH) which elicits a hyperemia reaction. As we have reported previously (7), the hyperemia reaction is elicited by prolan B in the ovary of infantile rats only if prolan A is present. Urine which contains only prolan A does not elicit the hyperemia AZT. During the last three years six to twenty-four hour hyperemia tests on different urines were run routinely concurrently with examinations in which the ninety-six hour original AZT rat method was employed. Attempts were further made to employ the hyperemia test quantitatively, since the assay of the chorionic gonadotropin is of fundamental importance in diagnosis of fetal death, mole, and chorio-epithelioma. In the course of this work, the surprising observation was made that rat unit values as determined by the hyperemia and the ninety-six hour test respectively, are only identical if an interval of eight to eleven hours is allowed in the hyperemia test between the injection

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and the examination. When time intervals outside this range were employed, serious discrepancies between the titers shown by the two methods were recorded. It was found in fact that the fluctuation of the rat unit value in the hyperemia test is a function of the time allowed between the injection and the examination.

The following experiments show how time affects the hyperemia AZT and define the relationship of different gonadotropin units to the hyperemia unit as a function of this time factor.

TECHNIC

Chorionic gonadotropin was in the form of a purified pregnancy urine preparation[1] which assayed 100,000 IU per Gm. The preparation was diluted with glucose to facilitate weighing. The diluted powder contained 1 IU in 12 mg. Suitable amounts of hormone were weighed out and dissolved in distilled water directly before each experiment to yield a concentration of 1 IU per cc.

The experiments were performed on a total of 700 infantile female rats, groups of 50 animals serving for an assay at each of the following time points: 1, 2, 3, 4, 6, 8, 10, 11, 12, 18, 20 and 24 hours. Controls of the estrus and luteinizing unit value of the gonadotropin solutions were run concurrently on groups of 10 infantile female rats.

DETERMINATION OF HYPEREMIA UNIT (HU)

A hyperemia unit (1 HU) is the minimum amount of gonadotropic hormone which elicits in the rat ovary, when given by single injection, a red color similar in tone to that of spleen, kidney, or liver. The hyperemia reaction is unequivocally demonstrable ten to twenty-four hours after the injection. Two to ten hours after the injection the ovary reaction is pink rather than red and can be definitely detected only by an experienced observer through reference to the swelling with which hyperemia is associated. The difficulty of making the reading at this time interval is evident from the work of Farris (1) who obtained paradoxical results using the two hour test. If the reaction is read at ten hours or later, the positive color reaction is unequivocally demonstrable where a dosage greater than the threshold dose has been given. We have gathered the impression that the hyperemia unit is less liable to variation than the estrus and luteinizing units. A hyperemia unit (1 HU) is therefore defined as the minimum amount of hormone which elicits a positive reaction in both ovaries of a rat in each animal of a group of 4. The superior accuracy of the hyperemia test over

[1] We are indebted to Dr. M. L. Tainter, Winthrop Chemical Co., Inc., New York, for a supply of Korotrin.

the estrus and luteinization tests is probably due to the fact that the former is based on a quantitative variation involving progressively varying degrees of redness, whereas the latter two tests are based on qualitative changes (follicle maturation, ovulation, luteinization) which conform to the "all or none" principle.

DETERMINATION OF ESTRUS UNIT (RU)

An estrus unit (RU) is the minimum amount of gonadotropic hormone which produces a positive vaginal smear reaction (keratinization) seventy-two to ninety-six hours after injection of a single dose into a 3–4 weeks old rat weighing 25–30 Gm. (3 & 6). Vaginal smears are made at intervals of seventy-two, eighty-four and ninety-six hours after the injection. The unit is defined as the minimum amount of hormone which elicits a positive reaction in 50 per cent of the injected animals.[2]

RESULTS

The results of our examinations may be stated briefly as follows. The hyperemia unit is a function of the length of time which is allowed to elapse between the injection of the hormone and the reading of the response (cf. table 1).

Comparison of the values of HU and RU in table 1 shows that 20 RU are necessary to evoke a positive hyperemia reaction in the ovary two hours after the injection. The effective dose is found to decrease as the time lapse between injection and reading is increased. It may be noted that when the time span is six hours 14 RU are still necessary, but that at eight hours 1 RU suffices. Before eight hours the ovarian reddening is less than maximal. After ten hours the characteristic red reaction, which can be recognized by the resemblance of the color of the ovary to that of spleen, kidney, or liver, is produced. At ten hours the effective hyperemia dose (HU) is actually smaller than 1 RU; at this time interval, 0.5 RU suffices to produce a

[2] For purposes of comparison the following additional units are given. Separate checks of these units were not undertaken in connection with the present study. In view of the practical importance of these units, expression of our results in these terms by conversions based on known data has seemed to us to be desirable.

MU (*mouse unit*) (3). A mouse unit is the minimum amount of gonadotropic hormone which produces a positive vaginal smear reaction (keratinization) 72–96 hrs. after injection into a 3–4 weeks old mouse weighing 8–12 Gm. The gonadotropin dose is subdivided into 6 injections. Vaginal smears are made at intervals of 72, 84 and 96 hrs. from the first injection. The unit is defined as the minimum amount of hormone which elicits a positive reaction in 50% of the injected animals.

IU. The International Unit can be calculated on the basis of the activity shown in the same test conditions by a standard preparation.

definite hyperemia effect. At terms greater than ten hours, the reaction becomes increasingly less sensitive, the number of RU which is required to produce a positive response increasing between twelve and twenty-four hours from 2 RU to 5 RU.

In routine examinations the following relations between the different units of chorionic gonadotropin were established:

1 IU = 2.5 MU = 5 RU (dose subdivided into 6 injections)
1 IU = 1.5 MU = 3 RU (single injection)

The quantitative relation of the different units is liable to variation depending on the strain of the test animal. In Germany, for example, the

TABLE 1. VARIATION OF THE HYPEREMIA UNIT ACCORDING TO THE LENGTH OF THE TIME LAPSE BETWEEN THE INJECTION OF GONADOTROPIC HORMONE AND THE READING OF THE RESPONSE

The RU was determined by parallel experiments. The IU was computed from RU with the help of a suitable calculation factor (0.2)

Time	2 h	3 h	4 h	6 h	8 h	10 h	11 h	12 h	18 h	20 h	24 h
HU:	1	1	1	1	1	1	1	1	1	1	1
RU:	20	16	15	14	1	0.5	1	2	3	4	5
IU:	4	3.2	3	2.8	0.2	0.1	0.2	0.4	0.6	0.8	1

senior author found the following relation: 1 RU = 1/5 − ⅛ MU (4, 5). It seems probable from a remark of Rakoff (2) that the hyperemia reaction, too, possesses, in different strains of rats, different degrees of sensitivity.

DISCUSSION

The experiments show clearly that a time factor is of great importance in the use of the hyperemia test for the quantitative assay of gonadotropin. We are convinced, however, that the hyperemia test is suitable as a basis of quantitative assay provided that a time factor table, like that which is presented in this paper, is accessible to the investigator.

The experiments show that the hyperemia test has maximum sensitivity if read ten hours from the time of the injection. At this time only 0.5 estrus units suffice to produce a positive hyperemia response. Need for care in interpreting the ten hour test is an obvious consideration. A ten hour test is positive not only to 250–500 RU per liter, a dose which also elicits a positive response in the usual test method (injection of 4 or 2 cc. urine and examination after 24 hours); it is found positive even with one-tenth of

this amount, i.e., as little as 25–50 RU per liter. The conclusion may be drawn that in a ten hour test for pregnancy a proper dose for injection is 0.2 and 0.4 cc. rather than 2 and 4 cc. of urine. Otherwise diagnostic confusion may arise between cases of pregnancy and conditions in which small amounts of luteinizing hormone occur in the urine. A 10 hour test with reduced urine dosages will be of advantage in the following circumstances:

1) determinations on rarely encountered toxic urine specimens;
2) determinations on small samples of urine;
3) tests in which rapid diagnosis has a high importance, e.g., extra uterine pregnancy.

Use of a ten hour test for ordinary routine examinations does not appear to be desirable, since its high sensitivity would lead, in cases of slight increase of urinary gonadotropin such as may occur even in a non-pregnant woman, to mistaken diagnoses of pregnancy. A high degree of reliability is afforded by the sixteen to twenty-four hour test which is, after all, quite rapid enough to meet the ordinary requirements of hospital and private practice.

SUMMARY

1. Chorionic gonadotropin elicits an early ovary reaction, anterior pituitary reaction II (APRII: Hyperemia reaction) in the infantile female rat. The hyperemia reaction does not fluctuate if the reading is carried out at a fixed time after the injection of the hormone. Large fluctuations are observed in the sensitivity of the test if the time span is allowed to vary between two and twenty-four hours. The hyperemia reaction is suitable for use in quantitative assay of gonadotropin provided that due allowance is made for the influence of the time factor.

2. Data summarized in table 1 show the variation of the sensitivity of the hyperemia test as a function of time span between the injection and the reading. The time-sensitivity curve has a parabolic form. Maximum sensitivity is found at ten hours when as little as 0.5 rat units is sufficient to elicit a hyperemic response. At eight and eleven hours the hyperemia unit equals the rat unit. The hyperemia unit (HU) rises progressively when the time span is varied below or above eight hours. At two and at six hours the HU is equal to 20 and 14 RU respectively. At twenty-four hours 1 HU is equal to 5 RU. The twenty-four hour test is four times as sensitive as the two hour test. The smaller reliability of the two and six hour tests as compared to the twenty-four hour test is explained by this finding.

3. The hyperemia reaction of the ovary at eight or less hours is pink. At ten hours or more, the ovary reaction is a deep red, similar to the color of spleen, kidney, or liver.

4. Using the twenty-four hour test, an accuracy of 99 per cent and better in pregnancy diagnosis was obtained. On the other hand, using the two and six hour tests to diagnose pregnancy, error rates of 31 per cent and 8 per cent respectively were experienced.

5. The ten hour test which affords the maximum sensitivity is recommended in the following special circumstances:

(a) examinations of toxic urine of which only small dosages can be injected into a test animal;

(b) cases involving suspicion of extra uterine pregnancy when speed of determination is a paramount consideration.

Disadvantages of the ten hour test as a routine examination are its extreme sensitivity and inconvenient time arrangement. Hence, the most suitable method for routine use is the eighteen to twenty-four hour test.

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1. FARRIS, E. J. Validity of the two-hour rat test for human pregnancy, *Am. J. Obst. & Gynec.* **48**: 200–207, (August 1944).
2. RAKOFF, A. E. Some recent laboratory procedures of importance in obstetrics and gynecology, *M. Clin. North America.* **39**: 1546–1556, (Nov. 1945).
3. ZONDEK, B. Ueber die funktion des ovariums, *Ztschr. f. Geburtsh. u. Gynäk*, **90**: 372–380, (1926).
4. ZONDEK, B., Weiters untersuchungen zur darstellung, biologie und klinik des hypophysenvorderlappenhormons (Prolan), *Klin. Wchnschr.* **8**: 157–159, (Jan. 22, 1929).
5. ZONDEK, B. *Hormone des ovariums und des hypophysenvorderlappens*, Berlin, J. Springer, 1931, 1st ed., p. 140: Vienna, J. Springer, 1935, 2nd ed. p. 230.
6. ZONDEK, B. and ASCHHEIM, S., Hypophysenvorderlappen and ovarium. Beziehungen der endokrinen drüsen zur ovarialfunktion, *Arch. f Gynäk*, **130**: 1–45, (1927)
7. ZONDEK. B., SULMAN, F. and BLACK, R. The hyperemia effect of gonadotropins on the ovary and its use in a rapid pregnancy test, *J.A.M.A.*, **128**: 939–944, (July 28, 1945).

TWENTY-NINTH ANNUAL MEETING OF THE ASSOCIATION

FOR THE STUDY OF

INTERNAL SECRETIONS

FRIDAY AND SATURDAY, JUNE 5–6, 1947

Haddon Hall, Atlantic City, New Jersey

GENERAL INFORMATION

Headquarters: Chalfonte-Haddon Hall, Atlantic City, New Jersey.

Rates are as follows:	Chalfonte	Haddon Hall
Single room with bath:	$6, $7, $9	$7, $8, $10
Double room with bath (without ocean view):	$8 and $10	$10 and $12
Double room with bath (side ocean view):	$12	$14
Double room with bath (ocean front)	$14 and $16	$16 and $18

Registration: Everyone attending the meetings is requested to register. A fee of $1.00 will be charged non-members of the Association. Membership cards should be presented when registering.

The Scientific Sessions: The Scientific Sessions will be held in the Viking Room of the Haddon Hall Hotel and programs will begin promptly on schedule. Papers presented at all meetings are planned for ten minutes and owing to the heavy schedule must be kept within this limit. Manuscripts of all papers should be submitted to the presiding officer or Secretary-Treasurer at the end of the presentation.

Annual Dinner: The Annual Dinner of the Association will be held on Friday evening, June 6, at 7:30 o'clock in the Rutland Room of the Haddon Hall, preceded by cocktails at 6:30 o'clock in the West Room. Secure tickets at time of registration.

Council Meeting: There will be a meeting of the Council on Thursday afternoon, June 5th at 2:00 o'clock, and a luncheon meeting on Friday, June 6th.

Business Meeting: The Annual Business Meeting of the Association and Election of Officers will be held at 4:30 p.m. June 7th, in the Viking Room of the Haddon Hall.

Local Arrangements: Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meetings.

Secretary-Treasurer: Henry H. Turner, 1200 North Walker Street, Oklahoma City 3, Okla.

PROGRAM

FRIDAY, JUNE 6

8:30 A.M. REGISTRATION

I. 9:30 A.M. *Viking Room.*

FULLER ALBRIGHT, presiding.

1. STUDIES IN CORPUS LUTEUM FUNCTION.
   J. S. L. BROWNE, J. S. HENRY, and E. H. VENNING.
2. PROGESTERONE THERAPY OF UTERINE FIBROMYOMATA.
   ALBERT SEGALOFF, JOHN C. WEED (*by invitation*) and WILLIAM PARSON.
3. DISTORTION OF THE SPIRAL ARTERY IN THE OVARY IN THE PRESENCE OF CORPUS HEMORRHAGICUM CYSTS AFTER ADMINISTRATION OF GONADOTROPHINS TO RABBITS.
   S. R. M. REYNOLDS.
4. GONADAL STIMULATION FOLLOWING THE ADMINISTRATION OF ANTIGONADOTROPHIC SERUM.
   HERBERT S. KUPPERMAN, R. K. MEYER, and J. C. FINERTY.
5. LIVER AND GONADAL CHANGES FOLLOWING THE ADMINISTRATION OF CARBON TETRACHLORIDE TO MALE RATS AND FEMALE GUINEA PIGS.
   B. KRICHESKY, S. J. GLASS, E. FURLONG, and M. FEINER.
6. CLINICAL EVALUATION OF DIENESTROL, A SYNTHETIC ESTROGEN.
   A. E. RAKOFF, K. E. PASCHKIS, and A. CANTAROW.
7. DIETHYLSTILBESTROL DIPALMITATE IN AQUEOUS SUSPENSION.
   S. CHARLES FREED.
8. THE EFFECT OF DIETHYLSTILBESTROL UPON ALLOXAN DIABETES AS RELATED TO FOOD INTAKE IN THE RAT.
   DWIGHT J. INGLE.
9. THE FACTOR OF PREVIOUS TREATMENT IN EXPERIMENTAL MENSTRUATION.
   DORIS H. PHELPS.
10. EXPERIMENTAL ALTERATION OF THE HUMAN OVARIAN CYCLE BY ESTROGEN.
    W. E. BROWN, J. T. BRADBURY, and A. F. JENNINGS.

PAPERS TO BE READ BY TITLE

11. STUDIES ON THE VARIATIONS OF BLOOD GONADOTROPHINS AND VAGINAL SMEARS DURING PREGNANCY IN CORRELATION WITH THE FETAL SEX.
    H. E. NIEBURGS, H. S. KUPPERMAN, and R. B. GREENBLATT.
12. ORAL ESTROGEN THERAPY DURING MENOPAUSE.
    ABBEY D. SELEY, DEBORAH BAUMGOLD, and SAMUEL VERNICK.
13. CORRELATION OF BASAL BODY TEMPERATURE CURVES WITH ENDOMETRIAL BIOPSY.
    A. R. ABARBANEL.

II. 2:00 P.M. *Viking Room.*

14. A RAPID METHOD FOR THE DETERMINATION OF URINARY "17-KETOSTEROIDS."
    I. J. DREKTER, S. PEARSON, and T. H. MCGAVACK.
15. FURTHER STUDIES ON THE METABOLISM OF THERAPEUTIC DOSES OF THE NATURAL ESTROGENS IN HUMAN SUBJECTS.
    B. F. STIMMEL and C. L. STEALY.
16. A SIMPLE QUANTITATIVE COLORIMETRIC TEST FOR ESTROGENS.
    HERMAN COHEN and R. W. BATES.

17. Urinary Steroid Balance in Virilism and Hypogonadism.
W. T. Salter, F. D. Humm, M. J. Oesterling and W. W. Engstrom.
18. The Metabolic Pathway of Estriol Production in the Organism.
Max N. Huffman and Arthur Grollman.
19. Color Reactions of the Steroids.
Herbert Jaffe, Babette Soloman and Robert H. Williams.
20. Chemical Assay of Urine for Adrenocortical Hormones in Endocrine and Non-Endocrine Diseases.
William H. Daughaday, Herbert Jaffee and Robert H. Williams.
21. Disappearance of Diabetes Mellitus Associated with Acromegaly Following Acute Mastoiditis and Basilar Meningitis.
T. P. Almy and Ephraim Shorr.
22. True Hermaphroditism: Report of a Case with an Ovotestis, and Endocrine Studies.
J. C. Weed (*by invitation*), A. Segaloff, Wm. Wiener (*by invitation*) and J. W. Douglas (*by invitation*).
23. Testosterone in a Case of Polyostotic Fibrous Dysplasia.
Rita S. Finkler and George M. Cohn.
24. Bilateral Arrhenoblastoma without Masculinization, Adenoma Testiculaire of Pick.
Minnie B. Goldberg and Alice F. Maxwell.

PAPERS TO BE READ BY TITLE

25. Social and Psychological Readjustment of a Pseudohermaphrodite under Endocrine Therapy.
Rita S. Finkler.
26. Some Observations on the Utilization of a Liquid Chromatogram Technique in the Colorimetric Estimation of Urinary Pregnanediol.
Benjamin F. Stimmel.
27. The Oral Use of Crude Adrenal Cortex in the Stimulation of Growth of the Face, Particularly the Condyle of the Mandible.
Francis M. Pottenger, Jr.

ANNUAL DINNER

III. 7:30 P.M. *Rutland Room, Haddon Hall*
Presentation of Ciba Award for 1947.
Presentation of E. R. Squibbs & Sons Award for 1947.
Presentation of Ayerst, McKenna & Harrison Fellowship for 1947.
Warren O. Nelson, *Chairman of the Committee on Awards for 1946–47.*
President's Address: Fuller Albright, Massachusetts General Hospital, Boston, Massachusetts.

SATURDAY, JUNE 7

IV. 9:00 A.M. *Viking Room.*
28. Chemical and Cytochemical Studies of the Rat's Adrenal Cortex Following the Administration of Pituitary Adrenocorticotrophic Hormone (Acth).
H. W. Deane (*by invitation*) and G. E. Bergner (*by invitation*).

29. Changes in Circulating Leucocytes Induced by Pituitary Adrenocorticotrophic Hormone (Acth) in Man.
    A. G. Hills (*by invitation*), P. H. Forsham (*by invitation*) and Clement A. Finch (*by invitation*). (*Introduced by* K. Emerson, Jr.).
30. Results of Administration of Anterior Pituitary Adrenocorticotrophic Hormone to a Human Subject.
    H. L. Mason, M. H. Power, E. H. Rynearson, L. T. Ciaramelli, C. H. Li, and H. M. Evans.
31. Metabolic Changes Following the Administration of Pituitary Adrenocorticotrophic Hormone (Acth) in Man.
    G. W. Thorn, F. T. G. Prunty (*by invitation*) and P. H. Forsham (*by invitation*).
32. Urinary Uric Acid-Creatinine Ratio Following Administration of Pituitary Adrenocorticotrophic Hormone (Acth) as a Simple Test for Adrenal Cortical Function.
    Peter H. Forsham (*by invitation*), F. T. G. Prunty (*by invitation*) and G. W. Thorn.
33. Further Studies on the Protective Power of Adrenal Preparations Against Bacterial Toxins.
    Lena A. Lewis and Irvine H. Page.
34. Effect of Testosterone upon the Excretion of Glycogenic Corticoids
    E. H. Venning and J. S. L. Browne.
35. Hepato-Renal Factors in Circulatory Homeostasis: XVII Relation of Adrenals to Formation of a Renal Vaso-Excitor Principle.
    B. W. Zweifach, E. Shorr, S. Baez, and S. Rosenfeld.
36. The Role of the Adrenal Cortex in Protein Catabolism Following Trauma.
    C. G. Toby and R. L. Noble (*Introduced by* J. B. Collip).
37. A New Hormone of the Adrenal Cortex.
    F. A. Hartman, K. A. Brownell, and J. S. Thatcher.
38. Studies on Obesity.
    R. H. Williams, W. H. Daughaday, W. F. Rogers, Jr., Herbert Jaffee, S. A. Asper, Jr., and B. Towery.

PAPERS TO BE READ BY TITLE

39. Pellet Therapy with Desoxycorticosterone Acetate in Adrenal Cortical Insufficiency.
    G. F. Koepf and (*by invitation*) R. Kibler.
40. Relationship of Sex and the Sex Steroids to the Adrenal Glands of Hamsters and Rats.
    H. S. Kupperman and R. B. Greenblatt.
41. Correlations of Biochemical and Histological Changes in the Adrenal Cortex in Various Types of Disease.
    W. F. Rogers, Jr., and R. H. Williams.

V. 2:00 p.m. *Viking Room.*

42. The Use of Hypertonic Saline Infusions in the Differential Diagnosis of Diabetes Insipidus and Psychogenic Polydipsia.
    A. C. Carter and Jacob Robbins (*introduced by* Ephraim Shorr).

43. Effect of Aqueous Testicular Extracts on Growth and Development of Spontaneous Mammary Tumors in the Aging Bitch.
F. X. Gassner.
44. Conjunctival and Corneal Lesions in Hypercalcemia.
J. E. Howard and F. B. Walsh.
45. A Secondary Sexual Character that Develops in an Organ Common to Both Sexes but Appears Normally only in Men. With a Discussion of the Relation of this Character to Endocrine Stimulation.
James B. Hamilton.
46. The Use of Methyl Testosterone and Testosterone Propionate in Premature Infants.
E. K. Shelton and J. S. Mark.
47. Equine Pituitary Gonadotrophin and Antihormone Formation.
J. H. Leathem and A. E. Rakoff.
48. Studies in Cases of Pituitary Tumors.
K. E. Paschkis, A. Cantarow, and A. E. Rakoff.
49. Carbohydrate Appetite of Normal and Hyperthyroid Rats as Determined by the Taste Threshold Method.
Curt P. Richter.
50. The Effect of Hypothyroidism on Menstruation.
W. O. Thompson, P. K. Thompson, and E. M. Jeppson.
51. Twelve Cases of Metastatic Thyroid Carcinoma Studied with Radioactive Iodine.
S. M. Seidlin, E. Oshry, and A. A. Yalow.
52. Goiter on an Iodine-Free Diet Grown by Hydroponics, and Excluding any Goiter Noxa.
J. F. McClendon and W. G. Foster.
53. Mitotic Activity and Wound Healing in the Corneal Epithelium of Rats Treated with Thiouracil.
W. Fleischman and I. A. Breckler.

PAPERS TO BE READ BY TITLE

54. Clinical Manifestations in Forty Cases of Myxedema.
D. Schwimmer, M. Vogel and T. H. McGavack.
55. Thiouracil in the Treatment of Hyperthyroidism Complicating Pregnancy and its Effects on the Fetus.
M. James Whitelaw.
56. The Permanency of Alloxan Diabetes and the Structure of the Pancreatic Islets Following Certain Experimental Proceedings.
Ralph G. Janes.

VI. 4:30 P.M. *Annual Business Meeting.*

Announcement

COUNCIL ON PHARMACY AND CHEMISTRY

OF THE

AMERICAN MEDICAL ASSOCIATION

The American Medical Association has entrusted the Committee on Therapeutic Research of the Council on Pharmacy and Chemistry with a fund to be expended in the promotion of investigations that may have therapeutic interest. The Committee invites applications for grants in this general field, which is conceived very broadly.

In the application there must be an adequate description of the research proposed. Since the grants from the Council may well be small in relation to the total cost of investigations aided, the Committee would appreciate information as to whether the proposed research is a continuation of investigation which has been supported previously by some research fund, and as to whether the work is to be supported in part by a grant or grants from other sources. If so, the Committee would like to know the name of the organization making the other grant and its amount.

Application should be in the hands of the Secretary of the Council, 535 North Dearborn Street, Chicago 10, Illinois, by June 1st. To expedite transmission to members of the Committee it is asked that six copies (five carbon copies) of each application be sent. Action will not be taken until after all applications are in.

STUART MUDD, M.D., CHAIRMAN
COMMITTEE ON THERAPEUTIC RESEARCH
COUNCIL ON PHARMACY AND CHEMISTRY